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PHYSIOLOGICAL REVIEWS

VOL. 22 APRIL, 1942 No. 2

BLOOD-BRAIN BARRIER

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In view of its ramifications in many branches of medicine (physiology, bacteriology, immunology and pharmacology), the problem of the blood-brain barrier is of more general interest than this term seems to indicate. A critical review of the widely scattered literature, therefore, may be of substantial help to those who are interested in the various aspects of the subject. This paper deals with the experimental evidence for the existence of this barrier, its localization, permeability to aniline dyes, toxins, viruses, antibodies and drugs, and with the physico-chemical factors controlling this permeability. The significance of these experiments for problems of pathogenesis and the physiological problem of capillary permeability will be discussed. It will be seen that on this broader experimental basis some fundamental questions can be definitively answered which were not decided by the rather one-sided consideration of experiments with aniline dyes.

It may be emphasized at the outset that this paper deals exclusively with the distribution of substances between blood and C-N-S. As will be shown, distribution between blood and cerebrospinal fluid (C-S-F) is an entirely different problem and remains outside the scope of this review.

I. *Fundamental facts.* The existence of a barrier between blood and brain was first suggested by the observation that certain substances, either colored or toxic, when injected into the vascular system, fail to produce any changes in the C-N-S, while they have marked effects after intracerebral, intraventricular or intrathecal injection. Such experiments have been reported with bile by Biedl and Kraus, with diphtheria toxin by Roux and Borrel, with potassium ferrocyanide by Lewandowsky and with trypan blue by Goldmann.

In another series of experiments it was shown that certain pathogenic agents reach the C-N-S exclusively by neural pathways. This was claimed for tetanus toxin by Meyer and Ransom and for the majority of neurotropic viruses by a large number of investigators. To what extent these fundamental observations actually prove the existence of a barrier between blood and C-N-S will be discussed in the following sections. Further experimental evidence pertaining to the subject will be presented.

II. *Older theories on the exchange of substances between blood and C-N-S.* v Monakow and Stern considered the C-S-F to be the nutrient fluid of the C-N-S. They, consequently, assumed that the exchange of substances between blood and C-N-S takes place exclusively through the C-S-F. If this were true, the permeability of the blood-brain barrier could be tested by an examination of the C-S-F. Actually a large number of clinical investigations have been carried

out on this basis. Although Spatz, (135, 136, 137) Riser, (21, 22, 23, 126) Walter and Morgenstern and Birjukow have raised serious objections to this theory it has been widely accepted and still finds its place in some otherwise excellent textbooks.

On the basis of general physiological considerations and experimental facts, however, this theory appears hardly acceptable. It is utterly unlikely that in an organ of the vital importance of the brain the capillaries should lack their chief uses, namely, mediation of the exchange between blood and tissue and adaptation of blood supply to functional needs.

In addition, Wesselkin, Friedemann and Elkeles (62) and Schmid invalidated the experimental foundation of this theory. Stern and Gautier claimed to have demonstrated a strict parallelism between the appearance of intravenously injected substances in the C-N-S and the C-S-F. The above mentioned authors, however, found independently that intravenously injected basic aniline dyes stained the brain intensely without appearing in the C-S-F. According to Friedemann and Elkeles this fluid is not colored even if the lateral ventricles and the cisterna magna are punctured at the moment when the exposed cortex becomes colored. Moreover, any transport of these dyes through the C-S-F could be ruled out by the observation that the grey matter was colored throughout the entire brain.

Experiments with acid dyes are of equal importance. As will be shown in the following section their intravenous injection leaves the brain entirely uncolored although, according to Wittgenstein and Krebs (161a) they appear in the C-S-F. This shows that the rate of absorption from the C-S-F into the blood is higher than that of their diffusion from the blood into the C-S-F. All these observations lead to the conclusion that the C-S-F has no appreciable part in the exchange of substances between blood and brain and that, consequently, as in other organs, this exchange takes place directly between blood and tissue.

It should be emphasized that the localization of the blood-brain barrier in the choroid plexus would be unjustified even if the exchange of substances between blood and brain took place solely by way of the C-S-F. It is obvious that in this case the cerebral capillaries would be impermeable to all substances circulating in the blood.

An entirely different conception of the exchange of substances between blood and tissues was advocated by Krogh and Ehrlich. Both authors deny the existence of any selective capillary permeability. According to Ehrlich (41) this exchange is controlled by chemical affinities. A similar opinion was recently voiced by King in a discussion of Goldmann's experiments with trypan blue. In the following sections it will be shown that the inability of certain substances to reach the C-N-S from the blood is actually due to a selective permeability of the cerebral capillaries. At the same time, with the aid of various methods, the permeability of these capillaries to aniline dyes, toxins, viruses, antibodies and drugs will be investigated.

III. *The permeability of the capillaries of the C-N-S to aniline dyes.* In 1887

Ehrlich (42) reported that subcutaneously injected acid aniline dyes (with very few exceptions) failed to stain the brain whereas a considerable number of basic aniline dyes gave positive results. However, the majority of them produced no staining. Mandelstamm and Friedemann (57, 58) found independently that this latter result holds true only if the dyes are injected subcutaneously. After intravenous injection almost all basic dyes stain the brain definitely. Ehrlich's negative results with acid dyes were confirmed by Goldmann, Spatz (138) and Friedemann (58).

The importance of these results for the theory of the blood-brain barrier is obvious. Concerning their interpretation, however, opinions are still divided. Only recently King has suggested that the negative results obtained with trypan blue are due to the fact that this dye has no affinity for any tissue elements in the C-N-S. In his opinion, trypan blue has a specific affinity for connective tissue which is absent from most of the brain.

Goldmann's well known second experiment was designed to rule out this explanation. It was shown that when introduced into the subarachnoid space, the dye stained the brain intensely. The experiment of Goldmann, however, is open to some serious objections. In the first place, it must be kept in mind that the volume of the C-S-F is only $\frac{1}{10}$–$\frac{1}{20}$ of that of the blood plasma. For this reason alone, when injected into the subarachnoid space, trypan blue should be 10 to 20 times more effective than after its injection into the vascular system. Actually *Lewandowsky* found that strychnine, although it certainly passes the capillaries of the C-N-S, was ten times more toxic by the intrathecal than the intravenous route. Moreover, according to Friedemann (56) and Bennhold, aniline dyes combine with the proteins of the blood plasma whereby their diffusibility is considerably diminished. Consequently the results of the subarachnoid and intravenous injections are not comparable.

To avoid this difficulty Friedemann (58) replaced the subarachnoid injection by an in vitro test. Small bits of brain were put into various dilutions of the individual dyes in blood serum and the minimal staining concentration (C_v) was determined. The minimal staining amounts of the intravenously injected dyes divided by the plasma volume give the minimal staining concentration in the blood plasma (C_B). Theoretically C_v should be equal to C_B if the cerebral capillaries were perfectly permeable. Table 1 shows that within the limits of experimental error this actually holds true for basic aniline dyes. As may be seen from table 2 the results are very different with acid dyes.

Table 2 shows that for acid dyes the values of C_B are high multiples of those for C_v.[1] This result would be inexplicable were the difficulty of acid dyes to reach the brain from the blood due only to their lack of affinity for nerve tissue. On the contrary, it shows that the capillaries of the C-N-S are either entirely

[1] According to Spatz (138) the faint staining obtained with large doses of intravenously injected trypan blue is a result of the dye content of the cerebral vessels. The real value of $\frac{CB}{CV}$ for trypan blue, therefore, would be considerably larger than that recorded in table 2.

impermeable or very little permeable to acid aniline dyes. These experiments with basic and acid dyes, therefore, are conclusive proof for the selective permeability of the capillaries of the C-N-S.[2]

Goldmann believed to have proven by his experiment that trypan blue has a definite affinity for nerve tissue but is prevented from reaching the brain by what is now called the blood-brain barrier. This explanation is probably not correct. Spatz (138) has shown that after a single subarachnoid injection of the dye the ganglion- and glia cells are not stained within the blue zone at the lower surface of the brain. Trypan blue reaches the nerve tissue merely by way of diffusion. These observations show clearly that chemical affinity is no prerequisite for

TABLE 1

The values of C_B, C_V and $\frac{CB}{CV}$ for some basic aniline dyes

DYES	MINIMAL STAINING AMOUNT (INTRAVENOUS)	C_B	C_V	$\frac{CB}{CV}$
	mgm.			
Bismark brown	2.5	1: 4,000	1: 2,500	1.6
Nile blue	1.0	1:10,000	1:10,000	1.0
Chrysoidin	1.0	1:10,000	1:10,000	1.0
Methylene blue	1.25	1: 8,000	1:10,000	0.8
Pyronin	2.0	1: 2,500	1: 5,000	0.5

TABLE 2

The values of C_B, C_V and $\frac{CB}{CV}$ for some acid dyes

DYES	MINIMAL STAINING AMOUNTS (INTRAVENOUS)	C_B	C_V	$\frac{CB}{CV}$
	mgm.			
Uranin	>80	>1:125	1:2,000	>16:1
Eosin	>40	>1:250	1:2,000	>8:1
Trypan blue	40	1:250	1:1,000	4:1
Congo red	80	1:125	1:2,000	16:1
Indigodisulphate	20	1:500	1:5,000	10:1

macroscopic staining of tissues, provided the dye is able to reach them. On the other hand, despite their affinity for nerve tissue pathogenic agents may be barred from reaching the C-N-S by the capillary endothelium. This will be demonstrated by the following experiments with toxins and neurotropic viruses.

IV. *The permeability of the capillaries of the C-N-S to toxins.* A conflicting literature illustrates the difficulties in establishing the central origin of toxic symptoms on the basis of clinical observations and physiological investigations.

[2] More comprehensive quantitative data concerning the staining of the brain by intravenously injected basic and acid aniline dyes have been reported by Friedemann (58) in a previous paper.

In addition, a specific action of a toxin on the C-N-S does not necessarily mean that it reaches it directly by way of the circulation. On the other hand, a toxin may fail to act on the C-N-S because it lacks any affinity for nerve tissue. The investigation of the permeability of the capillaries of the C-N-S, therefore, requires special methods which will be described in the following sections.

A. *Tetanus toxin.* In a series of ingenious experiments Meyer and Ransom claimed to have proven that tetanus toxin reaches the C-N-S exclusively by neural pathways. Recently, however, part of the experimental evidence on which this theory was based has been severely criticized by Horster and Whitman, Doerr, Seidenberg and Magrassi and Abel and his co-workers. Abel and more recently Doerr (29) arrived at the conclusion that, like other toxins, tetanus toxin reaches the C-N-S directly by way of the circulation and that the symptom of local tetanus is due to a direct action of the toxin on the muscle. Friedemann, Zuger and Hollander (70, 71) and Friedemann, Hollander and Tarlov have reinvestigated the problem with the aid of new methods. Concerning experimental details the reader may be referred to the original papers. The following results were obtained.

1. Intramuscularly injected tetanus toxin reaches the C-N-S even if any transport through the circulation is excluded by a large excess of circulating antitoxin.
2. Tetanus toxin reaches the C-N-S by way of the motor nerves.
3. Local tetanus is of central origin.

The third point is of particular importance for our problem. It is obvious that after exclusion of the peripheral origin of local tetanus this symptom could not be explained if tetanus toxin reached the C-N-S by way of the circulation. The capillaries of the C-N-S, therefore, are impermeable to tetanus toxin.

B. *Diphtheria toxin.* The question of the permeability of the cerebral capillaries to diphtheria toxin was first investigated by Bieling and Gottschalk. They injected large doses of toxin intravenously and determined the toxin contents of various organs by Roemer's method. No toxin was found in the brain. The almost complete absence of toxin from the liver, however, casts some doubt on the reliability of this procedure. We have reinvestigated this problem in a series of as yet unpublished experiments. They were based on an old observation of Roux and Borrel recently confirmed by Doerr and Kon and Bieling and Oelbrich. As mentioned before, the rat is almost immune to diphtheria toxin given by the ordinary routes while highly susceptible to its intracerebral injection. Roux and Borrel explained this observation on the assumption that in the rat the toxin is fixed by other organs and, thereby, deviated from the brain. Using Roemer's method for identifying the toxin in the blood, it was found that the toxin does not disappear more rapidly from the circulation of the rat than from that of the rabbit or guinea pig, which are highly susceptible to injection by ordinary routes. One and a half hours after the intravenous injection of 1 cc. of toxin, the blood of the rat still contained 80 intracerebral lethal rat doses. Dr. H. Zimmerman of New Haven examined the brains of our rats histologically. Even after the intraperitoneal or intravenous injection of 0.5 to 1 cc. of toxin no lesions were present, whereas the intracerebral injection of a single lethal dose

(0.005 cc.) was followed by marked pathological findings. All these experiments, taken together, leave little doubt that in the rat the capillaries of the C-N-S are impermeable to diphtheria toxin.

The question is more complicated in the case of the rabbit and the guinea pig. The following facts, however, seem to indicate that the impermeability of the cerebral capillaries to diphtheria toxin also holds true for the rabbit. Friedemann, Hollander and Tarlov have shown that in order to prevent local tetanus after intramuscular injection of the toxin, antitoxin is far more effective by the intraventricular than the intravenous route. Friedemann and Elkeles (59), however, in experiments on rabbits, found that the protecting dose of antitoxin against intravenously injected diphtheria toxin was exactly the same by the intraventricular and the intravenous routes. In connection with the tetanus experiment this result tends to show that after intravenous injection diphtheria toxin does not act on the C-N-S of the rabbit. Since, according to Caporali and Friedemann and Elkeles (59), diphtheria toxin is from 20 to 40 times more toxic by the intracerebral than the intravenous route, its affinity for nerve tissue is beyond doubt and its inability to reach the C-N-S from the circulation can be explained only by its failure to pass the capillaries of the C-N-S.

In view of these results the theory of Romberg, Paessler, Bruhn and Mueller that circulatory failure in the early stages of diphtheria is due to the action of the toxin on the vasomotor center in the medulla is no longer tenable.

C. *Botulinus toxin.* Physiological investigations of Dickson and Shevsky and Bishop and Bronfenbrenner have shown that botulinus toxin acts on the peripheral nerves. In conformity with these findings, Friedemann and Elkeles (65) arrived at the conclusion that the toxin does not reach the C-N-S from the circulation. One hour after the intravenous injection of 0.1 cc. of botulinus toxin into mice of 20 grams, 1 cc. of blood contained 1,000 intraperitoneal M.L.D. whereas the brain was found to be free from toxin. As a matter of fact Forssman has shown in interesting immunological experiments that botulinus toxin has no affinity for the C-N-S. The aforementioned experiments with trypan blue, however, have shown that, despite a lack of affinity, a colloidal substance may diffuse into nerve tissue unless it is prevented from doing so by an impermeable barrier. We, therefore, are led to the conclusion that the capillaries of the C-N-S are impermeable to botulinus toxin.

D. *Dysentery toxin.* After intravenous injection the toxin of the Shiga dysentery bacillus produces paresis in rabbits. The patchy and hemorrhagic character of the histological lesions in the C-N-S (Doerr and Seidenberg, 34), however, indicates that the capillary system is damaged by the toxin. It is impossible, therefore, at the time being to determine the permeability of the normal capillaries of the C-N-S to this toxin.

E. *Cobra venom, lamb dysentery and staphylococcus toxins.* These three toxins are discussed together because, contrary to the first four toxins, they act rapidly without any noticeable incubation period. This makes possible the application of two methods devised by Friedemann and Elkeles.

In the first method the brain of one rabbit, the recipient, is perfused by the

blood of another rabbit, the donor. After ligation of the vertebral arteries of the recipient both carotid arteries and jugular veins of the two animals are anastomosed. The toxins are injected either into the circulation of the donor or into the femoral vein of the recipient. The details of the method and some of its applications have been described by Friedemann and Elkeles (60, 61).

The second method is based on the observation of Friedemann and Elkeles (63) that adrenaline causes a 5- to 10-fold increase in the permeability of the cerebral capillaries to those substances to which they are already permeable (ethylurethane, ethylalcohol, paraldehyde, strychnine and alizarinblue-S). The impermeability to other substances (arsphenamine, trypanblue) remains unaltered. Consequently, adrenaline enhances the toxicity of those toxins which pass the capillaries of the C-N-S while it does not affect those which fail to do so. This method will be spoken of as the method of the auxoneurotropic effect.

Artificial perfusion of the brain was not applicable to cobra venom. The well known action of this toxin on the arterioles (Elliot) interrupted the perfusion at once. Adrenaline, however, reduced the rabbit intravenous lethal dose from 0.5 mgm. to 0.025 to 0.05 mgm. The capillaries of the C-N-S, therefore, are permeable to cobra venom (65).

Adrenaline also increased the toxicity of intravenously injected lamb-dysentery toxin[3] 4 times. In this case artificial perfusion also gave significant results. When the toxin was injected into the femoral vein of the recipient the cerebral reflexes (corneal, lip and tongue) disappeared at once. Obviously, it had reached the brain through anastomoses between the vertebral and spinal arteries. Both methods, therefore, led to the conclusion that the capillaries of the C-N-S are permeable to lamb-dysentery toxin (65).

Contrary to the results obtained with cobra venom and lamb-dysentery toxin Friedemann (58) found that adrenaline had no influence on the toxicity of intravenously injected staphylococcic toxin. The drug had even a slight protecting effect. It must be concluded, therefore, that staphylococcus toxin does not reach the C-N-S from the circulation. On the other hand the toxin was found to be 10 times more toxic by the intracerebral than the intravenous route. These results clearly indicate that the capillaries of the C-N-S are impermeable to staphylococcus toxin. On the basis of pharmalogical studies Burnet and Kellaway arrived at the same conclusion.

V. *The permeability of the capillaries of the C-N-S to neurotropic viruses.* It is now generally accepted that, if injected into tissues, the majority of neurotropic viruses reach the C-N-S exclusively by neural pathways. In the opinion of Hurst this indicates that the blood-brain barrier is impermeable to these viruses. His views, however, are not accepted by *Doerr* (28, 29). Actually the conclusions of Hurst would be convincing only if it could be shown that after peripheral injection these viruses circulate, at least for some time, in the blood stream. This certainly does not hold true for the strictly neurotropic viruses, namely, the viruses of poliomyelitis, rabies, and Borna disease. It is doubtful in the case of

[3] The lamb-dysentery bacillus produces diarrhea in newborn lamb. It is an anaerobic organism of the perfringens group.

herpes simplex. The permeability of the capillaries to all these viruses, therefore, can be tested only by their intravenous injection. From this point of view the entire literature on neurotropic viruses has been reassessed. The interesting material will be published elsewhere. In view of the limited space I must confine myself to the results. The evidence is conclusive or highly suggestive that the viruses of poliomyelitis, rabies, Borna disease, herpes simplex, louping ill, pseudorabies, St. Louis encephalitis, vesicular stomatitis, the neurotropic strain of yellow fever and equine-encephalo myelitis in mice and old guinea pigs are unable to pass the capillaries of the C-N-S. In the case of virus B, the existing experimental evidence is inconclusive.

Some neurotropic viruses pass the capillaries of the C-N-S but not in a physical sense. They infect the capillary endothelium and thus grow through the capillary membrane. This has been shown to hold true for equine encephalomyelitis in young guinea pigs and canine distemper.

The virus of fowl plague assumes a peculiar position. This is a pantropic virus but Doerr and Seidenberg (33), working with a highly virulent strain in chickens, have shown that the brain becomes infected early in the disease. It is very likely, therefore, that this virus passes the capillaries of the C-N-S of chickens. Other experiments of Doerr and Seidenberg (32, 35a) have shown that the same holds true for guinea pigs and mice.

Some observations seem to indicate that the ability of viruses to pass the capillaries of the C-N-S is in some way connected with their virulence. Working with a less virulent strain Lagrange arrived at the conclusion that the virus of fowl plague is unable to pass the capillaries of the C-N-S in the chicken. It has also been found that these capillaries may be permeable to a virus in one species, while impermeable in another. Experiments of Kleine and Moellers seem to indicate that in adult geese the blood brain barrier is impermeable to fowl plague virus. In adult mice the capillaries of the C-N-S are impermeable to the neurotropic strain of yellow fever virus. But they are apparently permeable in young mice (Theiler) and hedgehogs (Findlay and Clarke).

Summarizing we may conclude that in the ordinary laboratory animals and in a physical sense the capillaries of the C-N-S are probably impermeable to all neurotropic viruses if we exclude the virus of fowl plague because of its pantropic character.

VI. *The permeability of the capillaries of the C-N-S to antibodies.* According to the literature the majority of investigators seem to believe that the blood-brain barrier is impermeable to antibodies. This belief is mainly based on the following facts:

1. The unsatisfactory results of serum therapy in tetanus and poliomyelitis.
2. The absence of antibodies from the C-S-F in highly immunized animals.
3. The experiments of Roux and Borrel which showed that rabbits actively or passively immunized against tetanus toxin were not protected against the intracerebral injection of a single lethal dose of the toxin.

Freund, Weichsel and Salfeld, and Abel and Chalian, however, assume that the capillaries of the C-N-S are permeable to antibodies.

Since we have seen that the exchange between blood and C-N-S takes place directly through the capillaries, the second argument apparently is no longer tenable. The experiments of Roux and Borrel, however, need careful consideration. Friedemann, Zuger and Hollander (68, 69), therefore, have submitted them to an experimental analysis. These investigations started from the observations of Descombey and of Mutermilch and Salamon that guinea pigs actively or passively immunized against tetanus toxin were protected against the intracerebral injection of as many as 20 lethal doses of the toxin. Their explanation was that the blood-brain barrier is permeable to antitoxins in the guinea pig but impermeable in the rabbit. Experiments of Friedemann, Zuger and Hollander (68) with diphtheria toxin and antitoxin, however, showed definitely that this explanation cannot be accepted. It was found that in both animals 10 intracerebral lethal doses of diphtheria toxin were neutralized by exactly the same concentration of antitoxin in the blood. This discrepancy between the tetanus and diphtheria experiments can be explained in the following manner. The intracerebral lethal dose of diphtheria toxin is almost exactly the same for the rabbit and the guinea pig. The intracerebral lethal dose of tetanus toxin, however, is approximately 100 times greater for the rabbit than for the guinea pig. When equal absolute amounts of tetanus toxin were given intracerebrally to both species, both were protected by the same concentration of antitoxin in the blood. The difficulty in protecting rabbits against intracerebrally injected tetanus, therefore, is not due to the impermeability of the blood-brain barrier to antibodies but to the high combining power of the test dose of toxin in the rabbit. Since in the guinea pig 0.001 cc. of antitoxin protected against 10 lethal doses of the toxin it becomes apparent that both in the rabbit and the guinea pig the capillaries of the C-N-S are definitely permeable to antitoxins.

With the aid of skin test experiments Friedemann, Zuger and Hollander (66, 67) developed a new method by which the distribution of antitoxin between blood and tissues can be determined quantitatively. It was found that the antitoxin concentration in the intercellular fluid is approximately 1/20 of that in the blood plasma. The method is less accurate for the brain. It may be gauged, however, that the coefficient of distribution of antitoxins between blood and brain is somewhere between 1/20 and 1/60. Despite the permeability of the capillaries of the C-N-S to antibodies their intrathecal application, therefore, appears justified. As mentioned before, tetanus antitoxin is 80 times more potent in preventing local tetanus by the intraventricular than the intravenous route.

VII. *The permeability of the capillaries to proteins.* The experiments with antibodies are of interest in connection with the much disputed question of the permeability of the capillaries to proteins and the protein contents of tissue fluids. While Starling, Krogh, Landis and Peters consider the latter as low, Drinker and his associates believe that they approach that of lymph. Thus far, the protein content of the tissue fluid has been estimated only indirectly from that of the lymph or pathological exudates. As shown in our original paper

our method determines the antibody concentration in the capillary filtrate itself. There is every reason for believing that the distribution of antibodies between blood and plasma runs parallel to the distribution of globulins. Since the albumin/globulin ratio of the tissue fluid is probably a little higher than that of the blood plasma the total protein content of the tissue fluid may also be higher than that calculated from its antibody content. It may amount to 5 or 10 per cent of the protein content of the blood plasma, a figure which fits in satisfactorily with the results of Landis obtained with an entirely different method.

It should be emphasized, however, that our figure holds true only for the skin. In the tissue fluid of the liver the protein content is certainly much higher and in the C-S-F and the aqueous humor it is considerably lower. As mentioned before, the protein content of the tissue fluid in the brain is probably lower than in the skin.

VIII. *The permeability of the capillaries of the C-N-S to drugs.* Thus far no systematic pharmacological studies on the blood-brain barrier have been reported. Until recently it has been taken for granted that substances which act on the C-N-S are able to pass its capillaries. The investigations of Heymans, however, have cast some doubt on the validity of this assumption. He has shown that CO_2, cyanides, nicotine, lobeline, sulfides and acetylcholin influence the respiratory center only indirectly via the carotid sinus.

More reliance may be placed on the identification of drugs in C-N-S. Positive results were obtained with a considerable number of alkaloids and with the sulfonamides (Riser and Valdigné). It may be safely assumed, therefore, that these drugs pass the capillaries of the C-N-S.

On the contrary, McIntosh and Fildes, Rudolf and Bulm, Underhill and Dimick, Kolls and Youmans, Voegtlin and others found that these capillaries are impermeable to neoarsphenamine. After intravenous injection of this drug no arsenic or only traces of it were found in the C-N-S. Wallace and his associates found that in other organs the coefficient of the distribution of bromides, iodides and thiocyanates was the same as that of chlorides. Their content in the C-N-S, however, was much lower. The capillaries of this organ, therefore, are relatively impermeable to these drugs. The question of the permeability of the cerebral capillaries to magnesium salts is puzzling. Despite their narcotic action they cannot be identified in the C-N-S (Gensler, Mansfeld and Bosany).

As mentioned before, Lewandowsky found with the aid of a different method that the capillaries of the C-N-S are impermeable to sodiumferrocyanide. Using the same method, Friedemann and Elkeles (65) arrived at the same conclusion concerning Bayer 205 (Germanin). This trypanocidal drug is entirely atoxic by the intravenous route. Injected into the subarachnoid space it was found to be extremely toxic.

IX. *The relation between the ability of substances to pass the capillaries of the C-N-S and their electrical charge.* The investigations reported in the preceding sections have shown that the capillaries of the C-N-S are permeable to basic aniline dyes, cobra venom, lamb dysentery toxin, fowl plague virus, antibodies

and a great variety of drugs. They are impermeable to acid dyes, tetanus, diphtheria, botulinus and staphylococcus toxins, to most if not all neurotropic viruses (in a physical sense), potassium ferrocyanide, arsphenamine, Bayer 205 and relatively impermeable to iodides, bromides and thiocyanates. The striking differences within the individual groups obviously call for an investigation of the physico-chemical factors controlling the permeability of the capillaries of the C-N-S.

As far as non polar substances are concerned there is little doubt that lipoid-solubility or surface activity play an important rôle. The following considerations deal only with polar substances. It is a striking feature of our results that molecular size is apparently of little significance. The capillaries of the C-N-S are permeable to the colloidal antibodies, cobra venom, lamb dysentery toxin and even to the corpuscular fowl plague virus while they are impervious to highly diffusible acid dyes. Among basic dyes, the colloidal night blue and victoria blue pass the cerebral capillaries readily.

The clear cut difference between basic and acid dyes points to the importance of the electric charge. Although it will be shown in section XI that this concept is open to some theoretical objections, it was the lead in our further investigations. In the following paragraphs the electrical properties of toxins, viruses, antibodies and some microörganisms will be considered in connection with their ability to pass the capillaries of the C-N-S.

1. *Cataphoresis experiments with toxins.* As may be seen from table 3, Friedemann and Elkeles (65) found, in conformity with our hypothesis, that the ability of toxins to pass the cerebral capillaries is closely related to their electrical charge.

Table 3 shows that the negatively charged toxins (tetanus, diphtheria, botulinus and staphylococcus) do not pass the cerebral capillaries while positively charged cobra venom does. The result obtained with lamb dysentery toxin shows that substances which are isoelectric at the pH of the blood can pass the cerebral capillaries. Unfortunately the number of true toxins is limited. Nevertheless, the agreement between theoretical expectation and experimental facts is too striking to be dismissed as a mere coincidence.

2. *The electrical charge of viruses.* In table 4 are recorded the available data concerning the electrical charge of neurotropic viruses.

As may be seen from table 4 the negative electrical charge of these viruses is in conformity with their inability to pass the capillaries of the C-N-S. It is doubtful, however, whether this is a relationship of cause and effect. Obviously, the factor of size would explain the results as well. It must be remembered, however, that the size of the elementary bodies of some viruses does not exceed that of large protein molecules. Moreover, the cerebral capillaries are permeable to fowl plague virus. The electrical charge of this virus is of the greatest importance for our problem. According to Lépine (97) it is negatively charged over a range of pH 6.2–8.2. The paper, however, gives no information concerning the virulence of the strain used. Dr. A. Todd, of the National Institute for Medical Research, London, (personal communication) carried out

cataphoresis experiments with the highly virulent strain of fowl plague virus used by Doerr and Seidenberg. At the pH of the blood the virus was found to go neither to the cathode nor the anode. The peculiar electrical charge of fowl plague virus is indicated also by investigations of Doerr and Gold who reported that it is much more readily absorbed by the electronegative charcoal than other viruses and bacteriophages. This fits in with its ability to pass the cerebral capillaries. Further investigations along these lines with various strains of fowl plague virus are clearly indicated.

TABLE 3

Cataphoresis experiments with toxins

TOXINS	pH 8.2	pH 7.4	pH 6.2
Diphtheria	A	A	A
Botulinus	A	A	A
Tetanus	A	A	A and C
Staphylococcus	A	A	A and C
Lamb dysentery	A	O	C
Cobra venom	C	C	C

A = toxin goes to the anode; C = toxin goes to the cathode; O = no migration.

TABLE 4

Cataphoresis experiments with neurotropic viruses

VIRUS	pH	CHARGE	AUTHOR
Louping ill	7.3	Negative	Lépine (107)
Borna disease	6.6–7.4	Negative	Nicolau and Kopacowska (129)
Yellow fever	5.2–7.0	Negative	Hindle and Findlay
Herpes simplex	5.3–7.8	Negative	Nicolau and Kopacowska (126, 127)
Rabies	5.8–7.4	Negative	Nicolau and Kopacowska (128)
Poliomyelitis	6.9–8.0	Negative	Lévaditi and Lépine
Equine encephalomyelitis (Eastern strain)	Above 4.1	Negative	Finkelstein, Marx, Bridges and Beard
Vesicular stomatitis	6.6–8.4	Negative	Olitsky and Cox (personal communication)

3. *The electrical charge of antibodies.* The electrical charge of purified pneumococcal antibodies has recently been investigated by Felton, Felton and Kauffman, Chow and Goebel and Green, McKhann, Kupnik and Fakey. The isoelectric points ranged from pH 7.0 to 7.4 conforming with the fact that the capillaries of the C-N-S are permeable to antibodies.

4. *The electrical charge of drugs.* It is remarkable that all drugs to which the capillaries of the C-N-S have been shown to be impermeable, namely, neoarsphenamine, Bayer 205, the anions of the ferrocy-anides, iodides, bromides and thiocyanates are negatively charged. On the contrary, the alkaloids are elec-

tropositive substances. They act on the C-N-S and, at least, some of them have been definitely shown to pass the capillaries of the C-N-S.

5. *The electrical charge of spirochaetes and trypanosomes.* There is no doubt that treponema pallidum, the spirochaetes of recurrent fever (Buschke and Kroo) and trypanosoma gambiense and rhodense may pass the capillaries of the C-N-S. Omitting older investigations carried out with a questionable technique we wish to point to the careful cataphoresis experiments of Broom, Brown and Hoare with various strains of trypanosomes. It was found that some of them carried positive and others negative charges. With the same strain a change in the charge was observed after a relapse and after treatment with arsenicals. Thus far, no attempt has been made to correlate the electrical charge of these organisms with their ability to pass the capillaries of the C-N-S. Such investigations would be of great interest in connection with the much discussed problem of the existence of neurotropic strains of treponema pallidum (Lévaditi and Marie).

Brown and Broom made another interesting observation. They found that electropositive trypanosomes conglomerated with the negatively charged erythrocytes while electronegative trypanosomes failed to do so. A similar conglomeration of trypanosomes with the capillary endothelicum may be a prerequisite for their ability actively to invade the C-N-S.

These investigations on the electrical charge of aniline dyes, toxins, viruses, antibodies and drugs seem to indicate that the ability of substances to pass the capillaries of the C-N-S may be explainable on an electrochemical basis. In section XI some theoretical aspects of this concept will be discussed.

X. *The permeability of other capillaries to polar substances.* The experiments of Goldmann, Rous, Gilding and Smith, Singer (88) and Schulemann have shown definitely that the capillaries in other organs are permeable to acid aniline dyes. In the choroid plexus (Wittgenstein and Krebs, 161a) ciliary plexus (Fischer) and peritoneum (Engel and Kerekes) the capillaries are even impermeable to basic dyes. Moreover, there can be no doubt that in some tissues the capillaries are permeable to the negatively charged tetanus, diphtheria, botulinus and staphylococcus toxins. According to Wallace et al. the capillaries in all organs excepting the brain are perfectly permeable to the anions of bromides, iodides and thiocyanates.

Nevertheless, Wittgenstein and Krebs (161b) have shown that the capillary system as a whole, although permeable to acid dyes, is much more readily passed by basic dyes. They found that the latter disappeared far more rapidly from the circulation than acid dyes. Some colloidal acid dyes, for instance water blue, could be identified in the blood plasma for many weeks. The same holds true for the electronegative drug Bayer 205.

It appears, therefore, that the complete impermeability to electronegative substances is a peculiarity of the capillaries of the C-N-S. The difference between these capillaries and those in other organs, however, is more of a quantitative than a qualitative nature. This difference can be explained on a variety of assumptions. We wish to point to the fact that according to Overton (122)

the permeability to basic and the impermeability to acid dyes is characteristic of cell membranes in general. It would be tempting, therefore, to assume that in the capillaries of the C-N-S the exchange takes place predominantly if not exclusively through the capillary endothelium whereas in other organs the intercellular cement substance (W. Zweifach) may be of greater importance. Whether this hypothesis can be substantiated by histological methods remains for further investigations.

XI. *Theoretical considerations.* It must be admitted that our conceptions are at variance with Overton's theory on the permeability of cell membranes. According to this author cell membranes are impermeable to ions. The vital staining properties of basic dyes are not due to their positive electrical charge but to the lipoid solubility of the undissociated dye bases. At the Symposium on permeability at Cold Spring Harbor (1940), however, the majority of participants arrived at the conclusion that cell membranes are permeable to ions. Moreover, the experiments of Mandelstamm and Friedemann (58) have definitely shown that the ability of basic dyes to pass the cerebral capillaries is independent of their lipoid solubility.[4]

Our point of view is further strengthened by the discovery of Michaelis that dried collodium membranes are permeable to cations but impermeable to anions. Although not of a lipoid nature, therefore, they resemble the capillaries of the C-N-S.

An irreciprocal selective permeability to ions was described by Wertheimer in the skin of the frog. This membrane is passed by basic dyes only from the inner to the outer surface and by acid dyes in the opposite direction. Wertheimer explains these results by assuming an asymmetrical structure of the skin with a resulting electrical potential gradient through the membrane.

A selective irreciprocal permeability to ions would follow also from the well known theory of the Donnan equilibrium. Actually King has tried to explain Goldmann's experiments with trypan blue in this way. Since it has been shown in section IV that the cerebral capillaries are impermeable to acid dyes, the site of the Donnan equilibrium should be the capillary membrane itself. In this case, however, the cerebral capillaries should be permeable to acid and impermeable to basic dyes in the direction from blood to tissue. For at the pH of the blood the proteins are negatively charged and their concentration in the blood plasma is more than 20 times higher than in the tissue fluid.

In this connection mention must be made also of the extensive work of Keller and his associates which is entirely based on an electro-chemical conception. In his opinion the distribution of substances in animals and plants is controlled by electrostatic forces in the tissues. To adapt his theory to experimental facts Keller assumes that the electrical charge of aniline dyes in the tissues and the body fluids is entirely different from that in water. Keller and Singer have published a table in which aniline dyes are classified on the basis of their "bio-

[4] In the opinion of the author, the experiments of *Nirenstein* cannot be considered as evidence for the theory of lipoid solubility. On the contrary, they point very strongly to the importance of electro-chemical factors.

logical charges". A glance at their table shows that this classification is in no way related to our experimental results on the permeability of the cerebral capillaries. Although I agree with Keller in the electrochemical aspect of the problem of distribution, I cannot accept some details of his theory.

This discussion shows that the selective permeability of the capillaries of the C-N-S to electropositive or electroneutral substances can be explained by a variety of theories. The important question of the reciprocality of the permeability of the capillaries to polar substances will be discussed further in section XIII.

XII. *Some toxicological problems.* In the preceding sections we have seen that the capillary system as a whole is more permeable to electropositive than electronegative substances. The same holds true for the majority of cell membranes. A relationship between the electrical properties of substances and their toxicity, therefore, presents itself as a possibility. We cannot review in this context the entire field of pharmacology. Our surmise, however, finds support in the high toxicity of the electropositive basic aniline dyes, alkaloids, biogenic amines and ammonium bases and the relative atoxicity of the electronegative acid aniline dyes, neoarsphenamine, Bayer 205 and potassium ferrocyanide.

Regarded from this point of view, toxins are of particular interest. At first sight no relationship seems to exist between their electrical and toxic properties. Despite their negative charges tetanus, diphtheria and botulinus toxins are just as toxic as cobra venom and lamb dysentery toxin. The electrical character, however, is reflected in the rapidity of action. The three electronegative toxins act only after an incubation period of one or several days. Electropositive cobra venom and electroneutral lamb dysentery toxin, however, kill susceptible animals within a few minutes. In conformity with our theoretical expectation it has been found that the two groups of toxins differ markedly in the velocity with which they pass the capillary walls. After intravenous injection tetanus, diphtheria and botulinus toxins can be identified in the blood for many hours. Cobra venom and lamb dysentery toxin, however, leave the vascular system almost immediately. This follows from the fact that their lethal dose is considerably higher by the intra-arterial than the intravenous route. This has been shown by Straub for the toxin of an anaerobic bacillus probably closely related to lamb dysentery toxin and by us for cobra venom and lamb dysentery toxin (unpublished experiments). Apparently a large proportion of these toxins leaves the vascular system during a single circulation through the capillary bed. In addition, however, the rapid action of these toxins must be due to the velocity with which they pass cell membranes or are absorbed at their surface. Otherwise it would not be explainable that these toxins act rapidly also when injected directly into the brain (Friedemann and Elkeles 65).

Our explanation of the length of the incubation period of toxins, apparently, does not apply to the experiments with staphylococcus toxin which combines a rapid action with a negative charge at the pH of the blood. Cataphoresis

experiments of Friedemann (58) which cannot be described in detail, seem to indicate that it may be more correct to correlate the incubation periods of toxins with the ξ-potential than with the sign of the electrical charge. This would have the indubitable advantage of relating gradations of permeability to a graded electrochemical factor. Actually the ξ-potential of staphylococcus toxin was found to be low as compared with that of diphtheria toxin. It is intermediate between the ξ-potentials of lamb dysentery toxin and cobra venom on the one side and electronegative toxins on the other. If this conception can be substantiated by further experiments it would lead to the important conclusion that permeation of polar substances through the capillary walls is a cataphoretic phenomenon and, consequently, irreciprocal.

XIII. *The pathology of the blood-brain barrier.* Although we have seen that under normal conditions the C-S-F has no part in the exchange of substances between blood and C-N-S this may be different if the choroid plexus or the meningeal vessels are damaged. On the basis of the theories of v. Monakow and Stern even the mere presence of constituents of the blood and especially of proteins in the C-S-F might be detrimental to the C-N-S. Along these lines Weil and Kafka have advanced a theory of the pathogenesis of general paresis. They found, by the hemolysin method, that in this disease the C-S-F has a high protein content and considered this factor responsible for the degenerative changes in the C-N-S. Hauptmann elaborated a similar theory for the pathogenesis of tabes dorsalis. The now established fact, however, that the normal cerebral capillaries are permeable to proteins deprives these theories of their theoretical foundation.

An increased permeability of the capillaries of the C-N-S under pathological conditions has been reported only by Faber. He found that in monkeys infected with poliomyelitis virus the C-N-S was stained by intravenously injected trypan blue.

In young mice the capillaries of the C-N-S are apparently not entirely impermeable to electronegative substances. This was demonstrated with trypan blue by Behnsen and with the neurotropic strain of yellow fever virus by Theiler. The "Kernicterus" of the newborn may point in the same direction.

An increased permeability of the capillaries of the C-N-S was artificially produced by Froehlich and Zak with theopylline, by Friedemann and Elkeles (63) with adrenaline and by Duran-Reynals with testicular extracts (spreading factor). In the experiments with adrenaline and testicular extract the increased permeability concerns only those substances to which the capillaries of the C-N-S are normally permeable. Froehlich and Zak found that in the frog theophylline rendered the cerebral capillaries more permeable also to some acid dyes. The investigations of Barbour and Abel and Macht as well as our own experiments, however, have shown that in the frog the cerebral capillaries are normally permeable to a number of acid dyes. The observation of Froehlich and Zak that after the application of theophylline intravenously injected potassium ferrocyanide was found in the brain requires further analysis. We leave the question open whether theophylline makes the cerebral capillaries permeable to such substances to which they are otherwise impervious.

A traumatic breakdown of the blood-brain barrier has been performed by McCurdy and Evans, Macklin and Macklin, Mendel, Morgenstern and Birjukow and Broman. The traumatized area was found stained by intravenously injected trypan blue. Mendel and more recently King explain these results on a chemical basis. They assume that traumatized brain tissue has an affinity for trypan blue while normal tissue has not. Experiments with neurotropic viruses, however, make this explanation appear unlikely. Flexner and Amoss, Lennette and Hudson, Zwick, Seifried and Witte, Sawyer and Lloyd, Galloway and Nicolau, Burnet and Lush, and Webster and Clow have shown that the intracerebral injection of sterile saline, serum or starch renders animals susceptible to the intravenous, intraperitoneal or intracutaneous injections of a variety of neurotropic viruses which are otherwise innocuous. Since these viruses are extremely pathogenic for the untraumatized C-N-S these results can be explained only by the effect of the trauma on the permeability of the cerebral capillaries.

SUMMARY

Experiments with aniline dyes, toxins, viruses and drugs have definitely established the existence of a barrier between blood and C-N-S. This barrier is localized in the capillaries of the C-N-S which are endowed with a selective permeability. The choroid plexus, meningeal vessels and C-S-F are not concerned with the exchange of substances between blood and C-N-S.

The permeability of the capillaries of the C-N-S has now been determined for a considerable number of aniline dyes, toxins, viruses, antibodies and drugs. As far as the experimental evidence goes it seems to indicate that the ability of substances to pass the capillaries of the C-N-S is determined by their electrochemical properties. The cerebral capillaries are permeable to substances carrying a positive or no charge at the pH of the blood while they are impermeable to those carrying a negative charge. The permeability of the capillary system as a whole may be more correctly correlated to the ξ-potential than to the sign of the electrical charge.

The capillaries in the majority of organs are permeable to electronegative substances but they are more readily passed by the electropositive ones. The choroid plexus, ciliary plexus and the capillaries of the peritoneum are permeable to acid (electronegative) and impermeable to basic (electropositive) dyes.

The higher permeability of the capillary system as a whole to electropositive substances explains the greater toxicity of basic drugs and the rapid action of cobra venom and lamb dysentery toxin in contradistinction to the slow action of tetanus, diphtheria and botulinus toxins. These investigations have shown that toxins, viruses and antibodies can be used for the study of physiological problems, while, on the other hand, the physiological approach to bacteriological and immunological problems has led to some important results. The distribution of toxins between blood and C-N-S and differences in the lengths of incubation periods have been explained from the point of view of permeability and on an electrochemical basis.

In concluding this paper it must be emphasized that the term "blood-brain

barrier" is no longer entirely adequate. It does not take account of the fact that the problem is only part of the more general problem of capillary permeability and it places too much emphasis on its negative aspect. Nevertheless the term has been retained in the title, partly for historical reasons, and partly because the impermeability of the cerebral capillaries to a variety of pathogenic agents is important for many questions of pathogenesis which concern the C-N-S alone.

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CYTOLOGICAL ASPECTS OF SYNAPTIC FUNCTION

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It is not difficult to anticipate a phase of investigation of the synapse in which recent refinements in histological and histochemical methods, combined with the already highly developed neurophysiological techniques, may produce new data of great importance in our understanding of the mechanisms of synaptic function. Attempts at closer synthesis of physiological and histological data, however, although actively in progress,[2] are still rendered difficult by the obstacles encountered in adequately preserving and staining synaptic terminals in the nervous system. These difficulties not only hinder the determination of needed histochemical and histophysiological data, but have made even the amassing of purely descriptive morphological data a relatively slow process. Nevertheless, a not inconsiderable body of relevant anatomical facts exists which has not thus far been summarized and made conveniently available for those who are interested but who have perhaps no direct experience with histological material. One of the primary aims of this account will therefore be to emphasize certain aspects of the anatomy of the synapse which seem to the reviewer to require more attention in general considerations of synaptic physiology, and which may suggest specific problems for future investigation.

The physiological evidence bearing on particular aspects of synaptic function has been reviewed frequently in recent years[3] and will only partly fall within the scope of this discussion. In fact, only a small part of the evidence from anatomy can be summarized in this review, for the essence of the organization of the nervous system, and especially of synapses, lies in the complexity of arrangement of parts and the diversity of structure of the parts themselves.

For additional literature on the morphology of synaptic endings, the reader may be referred to the final summary of the eminent Spanish histologist, Ramón y Cajal (1934).

GENERAL HISTOLOGICAL DETAILS. Although half a century has elapsed since the pioneer histological studies of synaptic axon endings[4] the methods used in such studies have changed during that period only in the direction of refinement.[5] The controversy on the neuron doctrine and on the problems of protoplasmic and neurofibrillar continuity at the synapse, which often occupied earlier neuro-

[1] The author is indebted to Prof. R. W. Gerard for profitable discussions of this material, and for useful suggestions.

[2] Lorente de Nó, 1935, 1935a, 1938, 1938a, 1939; Young, 1939; O'Leary, 1940; Renshaw, Forbes, and Morison, 1940; Lloyd, 1941; and others.

[3] Forbes, 1922; Gerard, 1931, 1932, and 1942; Eccles, 1936, 1937, 1939; Gasser, 1937; Bronk and Brink, 1939; Rosenblueth, 1940; Nachmansohn, 1940; Bishop, 1941; Symposium on the Synapse, J. Neurophysiol., 1939.

[4] Ehrlich, 1886; Aronson, 1886; Retzius, 1889; Ramón y Cajal, 1893, 1896, 1903; Held, 1893, 1897; Berkley, 1896; Meyer, 1896, 1899; Auerbach, 1898; Huber, 1899.

[5] Bartelmez and Hoerr, 1933; Fedorow, 1935; Speidel, 1936; Bodian, 1937.

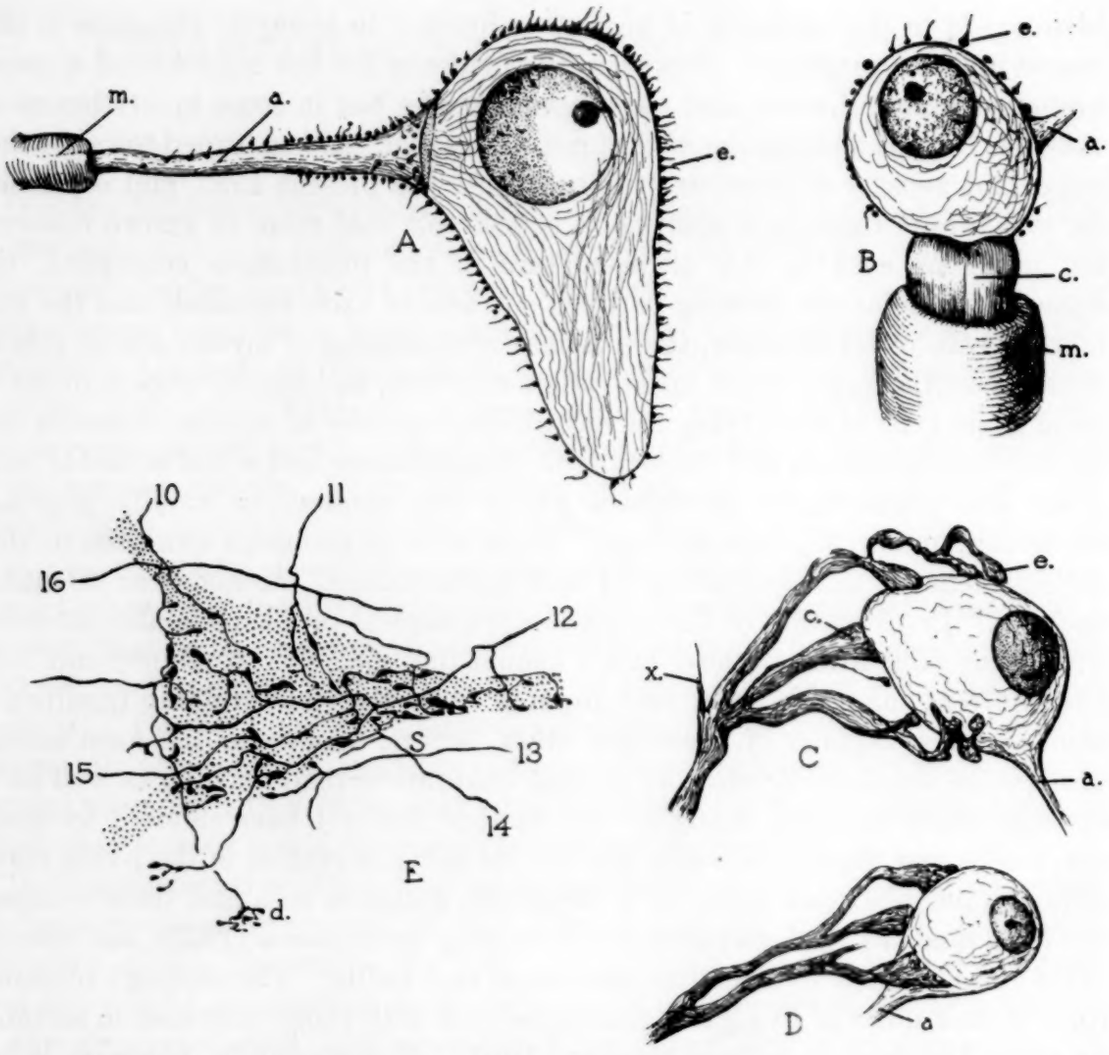
histologists to the exclusion of any other interest in synaptic structure is still waged in some quarters.[6] However, the evidence for the existence of a membranous synaptic barrier, not only in vertebrates but in some invertebrates as well, has become so voluminous that much investigation has turned toward other important aspects of synaptic structure. At the present time, and especially for purposes of functional analysis, it is desirable that more be known concerning problems such as the ultrastructure of the membranes concerned, the quantitative relations existing between surfaces of axon terminals and the surfaces of cells of termination, the intimate relationships of myelin and of glia to synaptic surfaces, the types, approximate numbers, and specific sites of origin of endings on cells of each type, the electrical properties of cellular elements and intercellular materials, and the localized concentrations and site of action of both drugs and physiological substances which may produce or modify neuronal excitation, under varying conditions. Some of these problems await the further development of microphysiological and histochemical methods for adequate analysis. Investigation of the purely morphological problems is also attended with great difficulties, because many axonal terminals are so minute and their post-mortem change is often very rapid.[7] The uncertain staining qualities of axon endings, because of these and other factors, constitute the most serious obstacle in the determination of normal anatomical relationships as well as of experimentally induced changes. No method has yet been devised to study nerve cells and their synapses in the central nervous system in the living state, although an important study of sympathetic ganglion cells and their synapses has been made in vital preparations of the frog by Fedorow (1935), and Speidel (1936) has studied living cutaneous axon end bulbs. The findings obtained from these studies of living material agree well with those described in suitably prepared histological sections (Bodian, 1940). Unfortunately, however, it has been impossible by present histological methods to demonstrate synapses with consistency in some centers where they are known to be present, and, in other centers, such as the mammalian thalamus, in which abundant terminal axon arborizations have long been known (Ramón y Cajal, 1911), details of the intimate relationship of the axon terminals to the cells of termination have only recently been described (Glees and Clark, 1941; Glees, 1941). These facts compel caution in generalizing from those synaptic bulbs which are more easily stained, although the great variety of the latter, coupled with their possession of certain characteristics in common, permits a tentative summary of their elementary characteristics as follows:

Synaptic endings as axon enlargements. Most axons and their collaterals terminate by means of protoplasmic enlargements (Ramón y Cajal, 1893) which serve to increase the terminal contact surface. The most easily and universally demonstrable type of synaptic axon ending is the endfoot or bouton (figs. 1 and 2). They are similar in form in all vertebrates,[8] as well as in invertebrates

[6] Nonidez, 1937; Nageotte, 1938; Boeke, 1940, 1941.

[7] Ramón y Cajal, 1903; Marinesco, 1904a; Bartelmez, 1915; Bartelmez and Hoerr, 1933; Fedorow, 1935; Speidel, 1936; Bodian, 1937, 1940.

[8] Ramón y Cajal, 1934; Phalen and Davenport, 1937; Bodian, 1937.



Figs. 1 and 2. A series of nerve cells showing various types of synaptic systems, and the distribution of axon endings on the surface of the terminal neuron.

Fig. 1. A. Large motor type cell from the reticular formation of the goldfish showing relatively uniform distribution of homogeneous boutons, *e*, on soma and proximal part of axon, *a*. From an 8 μ section, Mallory-azan stain. × 960. This type of synaptic system is the most common in the vertebrate nervous system and is characteristic of motoneurons and interneurons. One manner in which the synaptic knobs may be related to parent fibers is shown in E, a large interneuron from the spinal cord of a 15 to 16 day old cat; Golgi method. (Redrawn from Lorente de Nó, 1938.)

B. Cell of reticular formation of goldfish showing in addition to small boutons, *e*, a single large club ending, *c*, of a myelinated axon, *m*. Such club endings on small cells are in contact with a relatively large part of the cell surface. From an 8 μ section, Mallory-azan stain. × 960.

C and D. Two cells of the oculomotor nucleus of the goldfish, showing distinctive basket-like system of endings (knob and club-like units) derived from a single large branching axon. The general pattern of such endings on all cells of this nucleus is similar but the details of branching and distribution of endings on the cell surface differ in each case. From 15 μ section. Bodian stain. × 960. Similar synaptic systems are found in many centers in the nervous systems of vertebrates (see page 157).

a, axon of cell of termination. *c*, club ending. *e*, endbulbs or boutons terminaux. *m*, myelin sheath. *x*, collateral fiber.

(Sereni and Young, 1932; Young, 1939), and their size ranges vary from about ½ μ to 5 μ, and as large as 7 μ.[9] Nevertheless, such boutons, although repre-

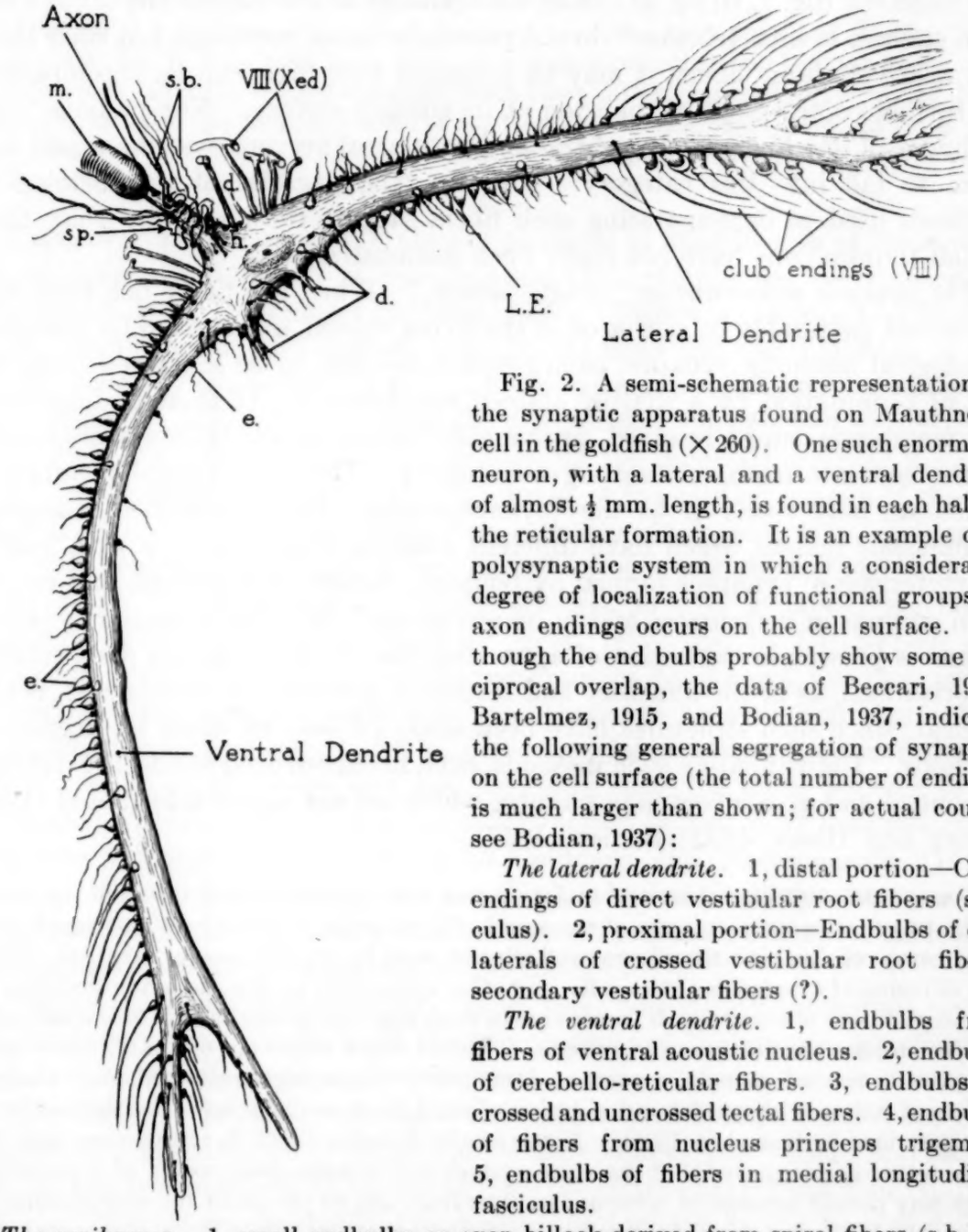


Fig. 2. A semi-schematic representation of the synaptic apparatus found on Mauthner's cell in the goldfish (× 260). One such enormous neuron, with a lateral and a ventral dendrite of almost ½ mm. length, is found in each half of the reticular formation. It is an example of a polysynaptic system in which a considerable degree of localization of functional groups of axon endings occurs on the cell surface. Although the end bulbs probably show some reciprocal overlap, the data of Beccari, 1907; Bartelmez, 1915, and Bodian, 1937, indicate the following general segregation of synapses on the cell surface (the total number of endings is much larger than shown; for actual counts see Bodian, 1937):

The lateral dendrite. 1, distal portion—Club endings of direct vestibular root fibers (sacculus). 2, proximal portion—Endbulbs of collaterals of crossed vestibular root fibers; secondary vestibular fibers (?).

The ventral dendrite. 1, endbulbs from fibers of ventral acoustic nucleus. 2, endbulbs of cerebello-reticular fibers. 3, endbulbs of crossed and uncrossed tectal fibers. 4, endbulbs of fibers from nucleus princeps trigemini. 5, endbulbs of fibers in medial longitudinal fasciculus.

The perikaryon. 1, small endbulbs on axon hillock derived from spiral fibers (s.b.) of the medial longitudinal fasciculus (from mesencephalic nucleus of the medial longitudinal fasciculus (?)). 2, knob endings of collaterals of crossed vestibular root fibers (VIII (xed)) near axon hillock (some uncrossed fibers (?)). 3, endbulbs of fibers in medial longitudinal fasciculus. 4, endbulbs of secondary acoustico-lateral fibers (?).

d—small dendrites. *e*—small endbulbs. *h*—axon hillock. *L.E.*—large endbulbs. *m.*—myelin sheath of axon of Mauthner cell. *s.b.*—bundle giving origin to spiral fibers. *sp.*—spiral fibers in region of "axon cap". *VIII (xed)*—crossed vestibular fibers giving rise to collaterals which terminate as small club endings. *VIII*—vestibular root fibers.

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[9] Sereni and Young, 1932; Hoff, 1932; Phalen and Davenport, 1937; Bodian, 1937; Barr, 1939; Gibson, 1940; Minckler, 1940.

senting the workaday prototypes of the synaptic axon endings, typify only one of several forms of such endings, and may occur alone or with other types on the same cell (fig. 1, B; fig. 2). The club endings of Bartelmez[10] (fig. 2) and certain endings in invertebrates[11] do not possess terminal swellings, but since these are relatively large fibers, it may be supposed that little can be accomplished by further enlargement of the axon at its contact surface. Non-bulbous "free endings" of fine fibers in the central and peripheral nervous systems would also seem to fall into this category. However, the vagaries of the histological methods used in demonstrating such fibers suggest the possibility that their actual terminations have not really been demonstrated.

The synaptic membrane or "synaptolemma." When the tissue has been well preserved, preferably by perfusion of the living animal, and stained by adequate cytological methods, synaptic enlargements are seen to be separated from the cell of termination by a sharply stained membrane.[12] At these endings only one membrane can be resolved, presumably because of the intimacy of contact of the separate neuronal limiting membranes. This appositional membrane, which we may speak of as the "synaptolemma" for convenience, separates cytoplasmic masses which have different staining properties as a rule, and is characteristic of synapses formed by boutons, club-shaped endings, and calyciform endings in vertebrates and in invertebrates. Whether a similar synaptolemma is present in the region of approximation of more complex axodendritic arborizations, as in the olfactory and cerebellar glomeruli of vertebrates, is not so clear, since such structures have been analysed only by silver impregnation methods. Unfortunately such methods, even in experienced hands, are difficult to control and give incomplete pictures which are not easily interpreted (Bartelmez and Hoerr, 1933; Bodian, 1937).

Young (1936, 1939) has described an interesting case of protoplasmic fusion of the axons of the two first order giant cells of the squid. These cells, however, are associated with other nerve cells on both the afferent and efferent sides by typical contact synapses. This case of fusion of axoplasms of two cells, and other cases cited by him, he has interpreted as secondary fusion of axons which "must always work together during the life of the animal." The fusion joins parallel synaptic systems, at points which would not ordinarily have been synaptically related, namely, a point of decussation of homologous giant fibers. Various degrees of intimacy of contact and of fusion of giant fibers at the point of decussation have been described by Hamaker (1898) in Nereis and by Johnson (1923) in crustaceans, and the latter author has suggested that this close contact, and in some cases fusion, of decussating fibers may permit passage of nervous impulses from one to the other for coordination of movements of the two sides of the body. In the cephalopods, Young (1939) states that this synapse-like relationship is apparently secondarily abolished by complete fusion, and other cases of fusion of parallel or homologous axons in invertebrates to form giant fibers have been described.[13] A somewhat analogous example of close association of parallel axons of homologous function within a single sheath has been described by Holmgren and van der

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[10] Bartelmez, 1915; Bartelmez and Hoerr, 1933; Bodian, 1937, 1940.

[11] Johnson, 1923; Stough, 1926; Young, 1939.

[12] See [10] and Young, 1939.

[13] Hamaker, 1898; Lewis, 1898; Johnson, 1923; Young, 1939; Cardot and Arvanataki, 1941.

Horst (1925) in the lungfish Ceratodus, but these writers doubt that the occasional points of fusion of these axons which they observed was not artefactual. The conduction properties of this interesting fascicle remain to be investigated.

Instances of protoplasmic fusion of dendrites have also been described (Dogiel, 1893; Glees, 1938), but it is doubtful that this is other than exceptional, and here the fusion does not abolish synaptically related surfaces. Such cases, although apparently breaking down the universality of application of some of the tenets of the neuron doctrine, do not in any way invalidate the evidence for the predominance of synaptic systems in all vertebrates, as well as in many of the invertebrates.[14]

Mitochondria at the synapse. On the proximal or afferent side of the synaptolemma an accumulation of mitochondria-like granules has been described (Bartelmez, 1915; Bartelmez and Hoerr, 1933). This was confirmed by Bodian (1937, 1940) for the club endings and also for large and small endings of the bouton type in the goldfish, as well as in the cephalopods (fuchsinophilic granules) by Young (1939). These granules are probably the neurosomes described by Held in 1897, although he failed to preserve the entire structure of the end-foot. It is noteworthy that in the "boutons" of the cardiac ganglia of the living frog, Fedorow (1935) has found that granules which stain with Janus green and with neutral red increase in number following stimulation of the preganglionic fibers. Concentrations of mitochondria in the synaptic cerebellar glomeruli have also been described by Held (1897) and by Ortiz-Picon and Pérez-Lista (1929). The function of these granules is unknown, but their aggregation near the synaptic membrane is a challenge to histochemistry. This aggregation might be interpreted as being correlated with a local secretion of acetyl choline or of choline esterase, especially since the latter appears to be associated with the surfaces at ganglionic synapses as well as at neuromuscular junctions (myosynapses), according to Couteaux and Nachmansohn (1940). On the other hand the aggregation of mitochondria might simply represent an anatomical signature of more general metabolic processes concerned in locally modifying the electrical properties of the membrane.

Neurofibrils at synaptic junctions. In all cases where a synaptolemma can be demonstrated, the neurofibrils of the two elements of the synapse are distinct, and do not cross the synaptic membrane.[15]

"Regulation" and regeneration of synaptic endings. Synaptic end-bulbs bear considerable resemblance to peripheral axonal endings, both sensory and motor, and all may be considered as stemming from the embryonic growth cone (Ramón y Cajal, 1890; Harrison, 1910). Such synaptic bulbs have also been described in cultures of embryonic chick brain.[16] The ability of peripheral axon endings in tadpoles to adjust to injury by forming new endings with different arborization patterns has led Speidel (1941) to suggest the possibility of a similar regulatory capacity of the central boutons. This notion is placed in serious questions by the fact that mature central axons possess notoriously slight capacities

[14] Schafer, 1878; Bozler, 1927; Hanström, 1928; Sereni and Young, 1932; Young, 1939; Woolard and Harpman, 1939; Ábrahám, 1940; Bullock, 1940.

[15] See [10], Ramón y Cajal, 1934; and others.

[16] Bauer, 1932; Mihálik, 1932; Grigorjeff, 1932.

for regeneration, and by the absence of clear evidence that mature central boutons ever retract, even during the period either of axon reaction of the terminal cells,[17] or of actual degeneration of the terminal cells (discussed by Ramón y Cajal, 1934). The tendency for boutons to adhere firmly to the terminal neuron, so as to resist separation by shrinkage in fixed preparations, has been noted frequently, and a high degree of stickiness has also been observed in teased preparations by Carpenter (1911). Regeneration of terminal boutons in sympathetic ganglia after degeneration following nerve section has however been described,[18] as well as coincident restoration of function in the case of the superior cervical ganglion.[19] In various reports on anatomical and functional regeneration of nerve fibers in the central nervous system, the fate of the terminal synaptic apparatus has not been considered (see Sugar and Gerard, 1940, for literature).

Degeneration of synaptic endings. Early suggestions as to the susceptibility of synaptic boutons to pathological states occurring, for example, in experimentally induced ischemia of the spinal cord (Marinesco, 1904b), and in rabies (Ramón y Cajal, 1904) have thus far led to no serious investigations of alterations of these structures in subtler pathological conditions. The difficulties of such investigations are obvious. The studies of experimental changes occurring in axon end-bulbs after axon section, although not as yet adequately exploited, have yielded more abundant fruit. Such studies, first carried out in sympathetic plexuses and ganglia,[20] have demonstrated conclusively the reality of the synaptic knobs as interneuronal junctions, and have opened up the possibility of histological controls for degeneration studies of synaptic physiology (Gibson, 1940). Although similar degenerative phenomena of synaptic boutons have now been described in the central nervous system by many investigators,[21] it remains to be seen whether the hoped-for precise determination of axonal connections by this method will be attained. Such a demonstration, to be rigorous, must surmount the difficulties presented by the great numbers of boutons on many nerve cells, often from many sources, the vagaries of the silver impregnation methods used in these investigations, resulting usually in incomplete staining of boutons in most instances, and the problem of distinguishing with absolute certainty the altered boutons from normal ones. Because of these difficulties, some of the claims made for this method have been criticized by Barnard (1940), who with Schimert (1938, 1939) has made clear the need for careful operative and histological control. Moreover, the validity of Hoff's findings (1932a, b) that pyramidal tract endings occur on motoneurons in the cat, and dorsal root endings do not, has recently been rendered questionable,

[17] Lawrentjew, 1934; Barr, 1940; Schadewald, 1940. Contra, Barnard, 1940.

[18] Lawrentjew, 1925, 1934; De Castro, 1930; Gibson, 1940.

[19] De Castro, 1930, 1936; Gibson, 1940.

[20] Nikolajew, 1893; Lawrentjew, 1924, 1925, 1934; De Castro, 1930, 1934, 1936; Kolossow, 1932; Kolossow and Sabussow, 1932; Kolossow, Sabussow and Iwanow, 1932; Baron, 1934; Fedorow, 1935; Fedorow and Matwejewa, 1935; and Gibson, 1940.

[21] Hoff, 1932a, b, 1935; Hoff and Hoff, 1934; Foerster, Gagel and Sheehan, 1933; Gibson, 1937; Schimert, 1938, 1939; Snider, 1936; Rosiello, 1937; Glees, 1941; Glees and Clark, 1941.

for example, by the contrary physiological findings of Lloyd (1941) and Renshaw (1940), respectively.

Transneuronal effects of axon section, consisting of cellular atrophy and extending beyond the synaptic junction, are implicit in the degeneration method of Gudden, and have in recent years been described as occurring after several months in adult individuals, especially in the lateral geniculate body following optic nerve section in primates.[22] Similar changes have been described in the spinal cord after root section by Foerster and Gagel (1934), who however were probably dealing with vascular injury. The transneuronal atrophy does not occur in all centers but probably only in those in which the severed afferent fibers form the bulk of the incoming nerve fiber supply. Ignorance of the mechanism of this atrophy is indicated by the suggestion that it may be considered as a "disuse atrophy". However, the increased irritability to chemical agents of denervated structures (Cannon, 1939) suggests a possible analogy to the "fatigue" atrophy of fibrillation in denervated skeletal muscle (Tower, 1939). Perhaps several factors determine the occurrence and degree of atrophy in deafferented neurons, just as in denervated muscle (Tower et al., 1941 a, b).

The synaptic environment. Since the region of the synapse is functionally of such great importance, it is necessary to inquire into the relationships of the various histological constituents of this region. Of the structures which may, by virtue of proximity and specific metabolism, affect the functioning of the synapse, the blood vessels, tissue fluid, neuroglia cells, and nerve fibers must be considered, but little can be said regarding the rôle of non-neuronal structures except that the close investment of synaptic structures by glial processes may be of some functional significance. An analogous investment of axon endings in muscle is suggested by the work of Noël and Pommé (1932), who consider the sole plate to consist of cellular elements enveloping the axon endings and continuous with and derived from the sheath cells of the terminating axon. Since Couteaux and Nachmansohn (1940) have found that in the region of the sole plates of mammalian gastrocnemius muscle an initial decrease of choline esterase after denervation is followed by the persistence of this substance in high concentration (60 per cent), it might be supposed that the supporting cells at effector endings play an important rôle in the function of myosynapses. This immediately suggests a similar rôle of glia cells in the functioning of neurosynapses, but although Couteaux and Nachmansohn have found a similar persistence of choline esterase in the superior cervical ganglion after preganglionic nerve section, the quantity involved was considerably less than in muscle and might have been localized within the nerve endings of undegenerated recurrent collateral fibers. Regardless of the interpretation of these data, however, it is evident that the conception of neuroglia cells as passive supporting structures is inadequate, since these cells may be of importance not only in the chemical economy of nervous tissue but also in the conditioning of its electrical properties. Indeed it is not inconceivable that the suggested varying importance of chemical as contrasted with bioelectrical mechanisms in synaptic transmission in differ-

[22] Minkowski, 1920; Balado and Franke, 1930; Clark and Penman, 1934.

ent nerve centers[23] may be dependent on functional differentiation of neuroglia cells or analogous cells in the peripheral nervous system.

Passing unmyelinated nerve fibers often thickly encompass a nerve cell or its dendrites, but such fibers have not been seen in actual contact with the surfaces of nerve cell bodies or of dendrites, except by means of synaptic swellings (L. de No, 1938). On some nerve cells, where endings are especially numerous, the passing fibers come no closer to the cell membrane than the diameter of the terminal bulbs, so that a fiber-free zone, not to be confused with shrinkage artifacts, can often be seen to surround the perikaryon or dendrite (Bodian, 1940). However, in some instances the fibers of the surrounding neuropil may press very closely upon a portion of a cell body or dendrite, and in some such cases one may see no terminal bulbs or any other type of synaptic structures upon this part of the cell surface (compare medial and lateral surfaces of ventral dendrite of Mauthner's cell, (fig. 2). In such cases, as also in the case of the cerebellar climbing fibers and "spiral" terminal fibers such as seen in the sympathetic ganglia[24] and around the Mauthner cell axon hillock (Bodian, 1940), it is conceivable that unmyelinated fibers may have a functional influence upon adjoining nerve cells or their processes. Whether in the vertebrate neuropil such influences of adjoining unmedullated fibers are of the nature of subthreshold excitability changes resulting in synchronization as described by Katz and Schmitt (1940) in crab nerves, or by Blair and Erlanger in the frog (1940), or are even capable of transmitting a nervous impulse (Jasper and Monnier, 1938; Renshaw and Therman, 1941) is conjectural. In the event of possible synapse by axonal apposition, such as the case of climbing fibers in the cerebellum, of granule cell collaterals of the cerebellum, and perhaps also of some of the fibers terminating on sympathetic ganglion cells, the existence of contact points or actual synaptic thickenings must seriously be considered (Lawrentjew, 1924; Ramón y Cajal, 1934).

Whenever pre-synaptic fibers are myelinated, whether these fibers are large or small, the myelin sheath terminates abruptly a short distance proximal to the contact surface[10] (see fig. 1, B, *m*). Furthermore, a differentiated myelin layer on the surfaces of the perikaryon, dendrites, or axon endings, has not as yet been detected by histological methods. This finding is in accord with the polarization optical studies of Chinn (1938), who found a thin lipid-protein sheath covering the dorsal root ganglion cells of the frog but no such layer over the perikaryon and axon hillock of the motor horn cells.

SPATIAL RELATIONS. Having considered the general aspects of structure in the region of the synapse, and the biological materials which may be involved in synaptic function, we come to a perhaps more formidable task. This involves dealing with the multiplicity of endings of axons in the nervous system, and their contiguous relations to the surfaces of other nerve cells. That this multiplicity of endings, with the accompanying complexities and diversities of interconnec-

[23] Bronk and Brink, 1939; Rosenblueth, 1940; Libet and Gerard, 1941.

[24] Arnold, 1863; Beale, 1863; Ehrlich, 1886; Retzius, 1889; Huber, 1899; Smirnow, 1900; Ramón y Cajal, 1906; Pitzorno, 1913, 1914.

tions, is of primary functional importance is axiomatic. Obviously, however, for purposes of analysis, one must reduce the complexities of construction and arrangement of neurons to one or another common denominator. In this instance the process will be that of limiting the discussion to morphological relations which might in any way affect the firing of single neuron units, since this is the ultimate problem of synaptic physiology. The discussion which follows will consist of two sections, the first dealing with monosynaptic systems, or neurons which might conceivably be excited by means of a single axon terminal, and the second dealing with the far more common polysynaptic systems, or neurons which must be excited by means of more than one axon terminal.

Monosynaptic Systems. A common postulate is that trans-synaptic excitation requires the disturbance of a given area of surface of a nerve cell by summation of activities of a number of closely spaced terminal membranes.[25] In the case of the small boutons this is suggested by the anatomical fact that these boutons are usually the terminations of collaterals of an arborizing axon, and that the fields of distribution of the boutons derived from two or more axons overlap. The arborization of a fine fiber near its termination could permit the excitation of several or many points on the surface of one or more cells of termination, and in the case of a particular cell of termination the ending of a single fiber by means of many boutons presumably would allow for disturbance of a larger surface area than could be effected by a single terminal knob.

The above assumption does not logically exclude the possibility of the existence of monosynaptic systems, that is, neurons receiving only one synaptic terminal. Nevertheless, McCulloch (1938) has questioned the justification for attributing the property of irreversible conduction to the individual synapse because of lack of evidence, first, for the existence of a monosynaptic system, and second, for effective excitation across a single synapse in either direction. This point of view fails to take into account considerable histological and physiological data of great interest. Johnson (1923) has described axo-axonal synapses of the segmental giant fibers in crustaceans which histologically are not unlike the large club-ending synapses in vertebrates.[10] A membrane is present between the two elements of the synapse in Cambarus, although he describes partial fusion in other species. When the membrane is seen, there is a constant staining difference on the two sides, and experiments showed no passage of degeneration, or of methylene blue, across the membranous barrier. A similar situation is described in the segmental giant fibers of annelids by Stough (1926, 1930) and by Young (1936). The former offers evidence for, and reviews the literature on, polarized conduction of the segmental giant fibers, and suggests a significant correlation between this polarization and the differential staining of protoplasms on opposite sides of the synaptic septa, since the darker staining protoplasm is constantly present on the proximal side of the membrane in both medial and lateral giant fibers. These fibers, upon which depend the longitudinal contractions of the earthworm (Yolton, 1923), conduct

[25] Eccles and Sherrington, 1931; Lorente de Nó, 1935, 1939; McCulloch, 1938; Young, 1939; and others.

in opposite directions as determined by isolated cutting of the fibers and stimulation of opposite ends of the body. Eccles, Granit and Young (1933), however, have found that impulses set up by induction shocks can pass in either direction along the giant fibers, presumably across the septa, and Young (1936) suggests that this reversible conduction across the membranes may be due to the similar dimensions of the membranes in contact. If this is true, and we are dealing with a monosynaptic system, it follows not only that reversibility is a property dependent on the relative dimensions of the contiguous synaptic membranes, but that in a monosynaptic system a single impulse may be effective across the synaptic barrier (see discussion of Lillie, 1936).

Young (1938, 1939) has shown in the stellate ganglion of the squid, in which the synapse involves a large contact surface, that a single impulse in the presynaptic fiber can set up a single impulse in the postsynaptic fiber. In this case the monosynaptic system involves nonsymmetrical synaptic surfaces, which, according to Young, may account for irreversibility at this synapse as compared with the symmetrical single synapses of the segmental giant fibers of the annelids.

Analogies in the vertebrate nervous system are not easily found, since most of the described neuronal systems are polysynaptic. However, the difficulty of demonstrating histologically the larger synaptic membranes in vertebrates may be partly responsible for this fact, smaller synaptic axon endings usually being associated with polysynaptic systems. The large club-endings on Mauthner's cell (fig. 2), first described by Bartelmez (1915) in Ameiurus, are here part of a polysynaptic system composed of many large and small endings on the dendrites, soma, and axon of Mauthner's cell (Bodian, 1937), but similar large club-endings on much smaller cells in the teleost tegmentum, described in the trout by Beccari (1934) and in the goldfish by Bodian (1937, 1940) present problems perhaps analogous to those of the monosynaptic systems in invertebrates since a single synapse involves a relatively large part of the cell surface (fig. 1, B). Such endings, which are transitional in form with cup-shaped endings, calyciform endings, and arborizing endings of large fibers in many centers in various vertebrates, have been described in the tangential nucleus of teleosts,[26] other tegmental cells of teleosts,[27] the trapezoid nucleus of mammals,[28] the oculomotor nuclei of teleosts,[29] the tangential nucleus of reptiles,[30] the ciliary ganglion of birds and of reptiles,[31] and the retina of vertebrates.[32]

Bodian (1940) has described the synaptic composition of some of the smaller cells in the tegmentum upon which single large club endings terminate (fig. 1, B). These endings have a diameter at the synaptolemma of about 7 to 9 μ, whereas the cells upon which they terminate, almost spherical in shape, have least and greatest diameters of about 14 to 16 μ. The surface of the soma of

[26] Ramón y Cajal, 1908, 1934; Tello, 1909; Beccari, 1931, 1934; Bodian, 1937, 1940.
[27] Beccari, 1934; Bodian, 1937, 1940.
[28] Held, 1897; Ramón y Cajal, 1934.
[29] Beccari, 1909, 1930; Bodian, 1940.
[30] Beccari, 1911; Segarra, 1926.
[31] Carpenter, 1911; von Lenhossek, 1911, 1912.
[32] Ramón y Cajal, 1932; Polyak, 1936, 1941.

such a cell, exclusive of dendrites, is roughly 500 sq. μ, whereas the surface of the club synaptolemma is approximately 50 sq. μ. Other synaptic endings, derived from axons other than those forming the club endings, are present in these cells in moderate numbers but these are extremely small as compared with the club-ending, perhaps of the order $\frac{1}{2}$ to 1μ. In short, such a cell may have roughly $\frac{1}{10}$ of the surface of its body in contact with the club-ending alone, and presumably a single impulse might be enough to excite the cell, regardless of the activity of the smaller endings. These club-endings are apparently derived from vestibular root fibers, and might be expected to dominate the synaptic field of the terminal cell when active. Such a polysynaptic system, in that case, might become a functional monosynaptic system, in which a single impulse might excite across the synaptolemma.

Polysynaptic Systems. A nerve cell, upon which two or more axons terminate, may be considered, along with the axonic terminals, as a polysynaptic system. Such systems represent the predominant types of synaptic aggregates associated with single neurons, especially in the vertebrate central nervous system, and probably represent the functional units with which one must deal in considering effective trans-synaptic excitation of such a single neuron. The synaptic composition of nerve cells varies greatly in different nerve centers, and to a lesser degree in any particular center, with respect to form, size, and numbers of synaptic endings, the distribution of these endings on the cell of termination (body, dendrite, axon), and the localization of different types of endings on specific regions of the synaptolemma[33] (see figs. 1 and 2). The analysis of the factors in synaptic transmission in such systems in terms of both temporal and spatial relationships, and of normal function, is a formidable task, but the fascination of the problems which can be approached with electrical methods applied to particular anatomical systems will no doubt ensure continued detailed studies of favorable systems.

Neurons with heterogeneous endings. Synaptic endings may be quite variable in numbers, sizes, and forms on nerve cells of different centers, and it is hard to imagine that this variety, with however a relatively constant pattern for the cells of each center, is not of functional significance. In addition to the simple types of endings of the bouton or club variety, each of which may predominate on any particular cell, more complex endings exist in many centers. Such are the basket types of endings, which may represent either multiple endings of the simple end-bulb type or combinations of club-like and bulb-like terminals derived from branches of a single fiber. This is clearly apparent in the case of the more complex endings on the cells of the oculomotor and trochlear nuclei in the goldfish (fig. 1, C and D). On these cells almost any assortment of end-bulbs, clubs, and even small calyciform endings may be seen, often derived from branches of a single axon. These endings were described by Beccari (1909, 1930), who found that cup-like endings on the cells of the oculomotor and trochlear nuclei of larval trout, derived from ascending fibers in the medial longitudinal fasciculus and in appearance much like the cup or calyciform end-

[33] Lorente de Nó, 1934, 1939; Bodian, 1937, 1940.

ings on the cells of the tangential nucleus,[26] enlarged and broke up during embryonic development into a complex of end-bulbs. A similar increase of complexity of axon terminal arborizations with age has been described by Nonidez (1941) in subendothelial venous receptors near the heart in dogs. The differentiation of terminating axons with respect to the degree of branching of the axon, the morphological variation of single terminating units, and the position on the cell of termination occupied by the axon terminals may not inconceivably be analogous in a limited way with the differentiation of peripheral axon terminals, especially those of the sensory systems, such as found in the skin. In this complex sensory organ, the bulk of present-day evidence, well summarized by Woolard, Weddell and Harpman (1940), favors the allocation of "specific sensory functions to morphologically characteristic nerve endings". These investigators furthermore find that with respect to cutaneous pain endings, "fibers heterogeneously distributed through a spectrum of sizes bear endings of a single morphological type". Similarly, it is the specific sensory ending which determines both the adequacy of a stimulus (threshold) and the duration of the sensory discharge (Adrian, 1930). It is also possible, however, that the morphological variation of the endings of a single axon on a single cell is of signifi-least and greatest diameters of about 14 to 16 μ. The surface of the soma of cance functionally chiefly with respect to the size of the terminal synaptic membrane (Beccari, 1930), or of its shape (Young, 1939), and to the numbers and arrangement of the endings. The size of the endings, as well as other cytological differences, such as the local physical constitution of the membrane may, for example, be correlated with the existence of specifically different synaptic thresholds of excitation.

The termination of a large fiber on a single cell by means of branching and terminal bulb formation is characteristic only of the endings in certain centers, as in the oculomotor nucleus of some teleosts, and has the effect of increasing the synaptic surface of even a large fiber. It may be significant that the details of the pattern of ending of the terminal bulbs of such large fibers on the teleost oculomotor neurons differ however on each cell (fig. 1, C and D). In the simple club endings of the type found on Mauthner's cell and on other cells of the teleost tegmentum, the terminating fiber, which may have a diameter as great as 7 μ, may end abruptly with little or no terminal swelling. The variability of the form and size of the endings in the central nervous system thus appears to be dependent largely upon conditions near the cell of termination during development, and secondarily upon the size of the terminating axon. The place of origin of the fiber has perhaps the least influence in determining the nature of the terminal axonic apparatus, as is also suggested by the regeneration experiments of Langley and others.[34] Thus, although the cells of various centers may carry quite diverse types of synaptic apparatus, in the form of simple endfeet, simple clubs, calyciform endings (tangential nucleus), or complex endings of the type found in the teleostean oculomotor nucleus, the endings are usually constant in type on the cells of any particular gray center. Mauthner's cell, which

[34] Langley, 1898; Harrison, 1910; Boeke, 1917, 1935; Baron, 1934; De Castro, 1934.

is a spectacular instance of a polysynaptic system in which localization of functional groups of axon endings occurs on the synaptolemma, is a striking exception to this rule, since several of these functional groups are characteristically and constantly differentiated with respect to size as well as form of endings.[8] Some reciprocal overlap of the functional groups of endings occurs, but the outstanding feature is the localization of some of the functional groups at comparatively distant regions of the enormous cell surface (fig. 2). This should offer an opportunity for testing experimentally the functional equivalence or non-equivalence of different portions of the synaptolemma (dendrites, body, axon hillock). This type of localization of endings of diverse types or origins on the synaptolemma is also characteristic of the Purkinje cell of the cerebellum, and also of other large cells with constant spatial orientation, and contrasts sharply with the overlapping arrangement of endings of diverse origin but of homogeneous types on the synaptolemma, described by Lorente de Nó as "partially shifted overlapping" (1934). A simpler version of heterogeneity of endings is found in the nucleus motorius tegmenti and in some of the vestibular centers of teleosts, on the cells of which one occasionally finds, in addition to the customary numerous endfeet, a large club ending[27] (fig. 1, B).

Neurons showing reciprocal overlap of homogeneous endings. When one examines most nerve cells one finds that the endings are relatively uniform in type. Furthermore, in preparations in which more or less complete preservation and staining of endings has been attained, these endings tend to be relatively constant in number, as well as in form and distribution, on cells of the same functional center.[35] Although in some preparations one finds cells with either no endings, one ending, or very few endings, this is usually an indication of incompleteness of preservation or staining, as can be seen by comparison with better preparations. Yet on some of the smallest nerve cells in many locations it has not been possible to demonstrate contact terminals of any kind, as in the substantia gelatinosa Rolandi. Whether this indicates a deficiency of the histological methods, due perhaps to the difficulty of adequately preserving extremely minute endings, or whether synaptic functions are mediated in these cases without benefit of contact terminals, is not known. The large cells of the "motor" type in the vertebrate tegmentum and cord are covered principally by terminals of the endfoot type, which are usually so numerous in favorable material that an accurate estimate of their numbers is impossible. Estimates on mammalian motoneurons run as high as 1200–1800 for the cell body alone,[36] and as high as several thousand on cortical pyramids (Lorente de Nó, 1933). When large numbers of endfeet or small club-endings are present on the perikaryon, the inter-distances between endings are rarely greater than the diameter of the endings themselves (Bodian, 1940). Hoff (1932) found that as many as 8 boutons per 100 μ^2 may occur on the anterior horn cell of the cat, but Barr (1939) has estimated that an average of 16 boutons per 100μ^2 occur on cat motoneurons (38 per cent of the cell surface), and Minckler (1940) finds on

[35] Ramón y Cajal, 1934; Bodian, 1937; Minckler, 1940.

[36] Barr, 1939; Minckler, 1940.

human motoneurons as many as 23 terminals per 100 μ^2, and 14.5 per 100 μ^2 on cells of the sensory column in the cord. It is important to emphasize these figures, since it has been frequently assumed that the amount of surface of a nerve cell covered by synaptic terminals is relatively small in proportion to the total surface of the cell. On the cells of the nucleus motorius tegmenti the endfeet have their greatest concentration on the cell body, and sometimes are seen to be more widely spaced on the proximal parts of the dendrites. However, in general, the endfeet are more satisfactorily demonstrated on the cell body than on the dendrites.

In Ramón y Cajal's comprehensive final survey of the anatomical literature on synapses (1934) one finds no mention of axo-axonic synapses, although descriptions of such synapses were available (Beccari, 1920) and have since been amply verified.[37] Such synapses, in the form of terminal endfeet, are frequently seen on the axon hillock of mammalian cells, and in the case of large axons as far out on the axon as the axon neck or even the beginning of the myelin sheath. When present on the proximal part of axons, the endings are usually more widely spaced than on the perikaryon. In the case of Mauthner's cell at least the site of origin of the parent fibers of the endfeet on the axon hillock and axon neck is different from that of those fibers which terminate on other parts of the cell. In the functional interpretation of the presence of endfeet on axons of cells the possibility must be considered that impulses which impinge on the axon may have the same effect as artificial antidromic stimuli. Coghill (1934) has suggested that endings in this location may have an inhibitory function, and this hypothesis has been elaborated in greater physiological detail by Gesell (1940), and by Gerard (1941a).

The endfeet, in addition to being closely spaced on the tegmental cells, are rather evenly distributed, as can best be seen in tangential sections of the cell surface. The appearance of the smallest endfeet on many of the tegmental cells, with respect to their size, form, staining qualities, and distribution on the cell membrane, closely resembles that of the dendritic "thorns" of the cerebellar Purkinje cells (Bodian, 1940) which were first interpreted as endfeet by Held (1897).

The heterogeneous synaptic systems illustrate the morphological difficulties encountered in any assumption as to the functional equivalence of all types of synaptic endings. For experimental purposes, however, such an assumption may be valid for certain homogeneous polysynaptic systems, that is, neurons bearing endings of one morphological type. Such systems have been analysed in detail by Lorente de Nó,[38] and appear to be characteristic of mammalian motoneurons and interneurons. It has been known since early times that at least in many systems of endings of homogeneous sizes and forms, an overlapping distribution of endings derived from arborizations of two or more parent fibers occurs. This reciprocal overlapping described in some detail by Lorente de Nó (1934) is of considerable significance in any interpretation of synaptic function,

[37] Coghill, 1934; Bodian, 1937, 1940; Young, 1939; Barr, 1939.

[38] Lorente de Nó, 1933, 1934, 1935, 1938, 1938a, 1939.

and it is highly likely that it is a general phenomenon as regards synaptic systems consisting of numerous homogeneous endings. Whether overlapping or coextensive aggregates of endings of different fibers on a single cell may influence each other mutually (summation, inhibition) is problematical.[39] Analogous overlappings of coextensive homogeneous nerve endings in the cornea apparently have no such mutual influences (Tower, 1940; see also Weddell, 1941, 1941a) but this is perhaps not a fair comparison. Whether the caliber of the arborizing telodendria of a single terminating axon, the size of the boutons, and their spacing on the cell of termination affects the timing of the components of a single impulse as dispersed by the terminal arborization of the axon, is also conjectural.

AUTONOMIC GANGLIA. The synaptic structure of autonomic ganglia is of special importance because of the ready availability of these centers for experimental investigation. Unfortunately, although the incident impulses to the ganglion as a whole may be fairly homogeneous, the actual synaptic composition in relation to any particular cell may be complex. Anatomically there is no evidence for any fundamental difference between sympathetic ganglia and central nuclei with respect to the structure and arrangements of synaptic systems on single cells or cell groups.

The endings on sympathetic ganglion cells were first clearly described in 1886 by Ehrlich, who noted the spiral course of some of the incoming fibers[40] before they arborized around the cells of the ganglion and terminated by means of small terminal swellings. These findings have been essentially corroborated by many workers since that time (see Huber, 1899) and additional important details added (Lawrentjew, 1924). Huber noted that several fibers may contribute endings to a single cell in mammalian ganglia and this has often been confirmed. Likewise, a single entering fiber may contribute endings to several cells in the ganglion (Smirnow, 1900). The presence of collaterals of axons of sympathetic ganglion cells has often been described,[41] and Lawrentjew has confirmed this finding by degeneration experiments (1924). He has also described arborizations of such collaterals which may contribute a group of several end-swellings to as many as four neighboring nerve cells (1914). Such findings suggest the possibility of the existence of "self-re-exciting chains" of neurons, minus interneurons, in sympathetic ganglia, which could perhaps partly account for the prolonged excitatory state described in such ganglia following multiple preganglionic volleys.[42]

In addition to the spiral and non-spiral entering fibers, and recurrent collateral fibers, all of which terminate by means of a few or many terminal swellings, often localized to one pole of the cell, Lawrentjew (1924), confirming Ramón

[39] See interesting evidence of spatial overlap and spatial summation in crayfish (Prosser, 1940).

[40] Spiral fibers associated with sympathetic ganglion cells were seen as early as 1863 by Beale and by Arnold.

[41] Ramón y Cajal, 1893; von Lenhossek, 1894; Dogiel, 1899; Smirnow, 1900; Lawrentjew, 1914, 1924.

[42] Bronk, Tower, Solandt, and Larrabee, 1938.

y Cajal (1906), has described fine incoming fibers which course along the dendrites, much like the climbing fibers of the cerebellum, and which possess collateral thickenings which form contact terminals on the dendrites. The origin of these fibers is unknown. Still other types of endings, of the club and basket type, perhaps of special functional significance, have been described in the ciliary ganglia of birds and reptiles,[31] which could perhaps be studied experimentally.

It is obvious that peripheral synaptic ganglia, which are not only convenient for functional studies, but are also anatomically comparable with central synaptic areas, are strategic areas for further analyses of synaptic physiology.

RÉSUMÉ

The large numbers of endings on most nerve cells appear to have but one logical significance, namely, that most of them at any one time are concerned with maintaining a subliminal state of excitability,[43] which may or may not reach the threshold level of somatic potential, depending (perhaps, in some cases, predominantly (Tower, 1937)) on the occurrence of effective discharges from the functional center dominating the phasic activity at any particular time. This functional anatomical concept, and that of self re-exciting chains of neurons and collaterals, are corollaries of the general concept of functional cell-chain systems, and fit in well with the existence of morphological heterogeneity and overlapping distribution of endings on neurons of termination. How far, and in precisely what manner, the functioning of such systems can be influenced by more diffusely acting forces, such as chemical substances or potential fields,[44] remains to be seen.

Furthermore, the temptation and necessity to create diagrammatic generalizations should not be allowed to obscure a fact which is most relevant for experimental investigation, namely, the great anatomical variability of particular synaptic systems. It is with particular systems that the experimenter must deal, and in the interpretation of data obtained from particular systems generalized diagrams are often misleading. The understanding of the specific characteristics of the anatomical set-up being used in particular physiological experiments is essential for the interpretation of the results, but, as often as not, physiological experiments reveal the fact that we are still ignorant of many relevant anatomical relationships.

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ORGANIC CHEMICAL INDUSTRIAL HAZARDS TO HEALTH

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A survey of industrial hazards to health caused by organic chemicals, as presented in the following pages, has necessarily to be restricted to the essential compounds; and for detailed information, whenever possible, references will be given to reviews on the various topics. For this reason the discussion will deal with chemical groups rather than with individual compounds, and, in each of these, their industrial use, the general character of their toxic action, and the general trend of the toxic properties within such groups will be analyzed. The arrangement according to chemical groups instead of in respect to toxicologic effects, such as irritants, blood poisons, circulatory poisons, nerve poisons, etc., appears to be advantageous because such classification avoids overlapping and will give a better basis for the appraisal and further study of such toxic substances as may gain importance from the industrial point of view. For this reason the subject will be arranged according to three main groups: the aliphatic hydrocarbons, the aromatic hydrocarbons, and the naphthalene derivatives; and in each the compounds produced by the introduction of certain radicals, such as hydroxy-, amino-, nitro-, and others, will be discussed.

A. The aliphatic hydrocarbons and their derivatives. 1. The *aliphatic hydrocarbons* may be subdivided into the following groups: Paraffins, olefines, diolefines, acetylenes, cycloparaffins and unsaturated cycloparaffins. The lower members of these groups with 1 to 4 carbons are gases at ordinary temperature; those with 5 to 15 in the paraffin, to 18 in the olefine, and to 6 in the ring of the cycloparaffin series are liquids; and those of higher carbon content are solids. The liquid aliphatic hydrocarbons are constituents of petrol ether, benzine and coal oil. But the composition of these solvents is not uniform and the toxicological properties of different brands of benzine differ quantitatively and qualitatively, especially if contaminated with aromatic hydrocarbons. Some of these hydrocarbons, such as cyclohexane and its homologues, are used in pure form; and others, such as benzine and coal oil, are used in mixture. They are used extensively as solvents in the chemical, lacquer and related industries.

The *paraffins* of the general formula C_nH_{2n+2} have narcotic properties which increase from hydrocarbons with one carbon to hydrocarbons with eight carbons in the chain. It appears that with some members the margin of safety between narcotic and toxic concentration is very small, and that hydrocarbons with straight chains are more toxic than those with branched chains containing an equal number of carbons. The liquid members of this group cause irritation of the nerve endings in skin and mucous membranes, increasing in intensity up to hydrocarbons with seven carbon atoms.

Similarly, the narcotic and toxic actions of *olefines* of the general formula C_nH_{2n} increase with the molecular weight and in inverse ratio to their solubility in water and in direct ratio to their solubility in oil. In opposition to the be-

havior of the corresponding paraffins, olefines with more than four carbons in the chain cause increased reflex excitability, and those with more than three carbons are said to affect the circulation also, partly by stimulation of the vagus. It appears, further, that some of these compounds may cause injury of the liver, whereas the irritant effect appears to be slightly less marked than with the saturated hydrocarbons.

Introduction of a second double bond yields *diolefines* and increases the narcotic action, and the same holds true for the introduction of a triple bond, as in acetylene.

The *cycloparaffins*, which represent ring structures containing from three to six carbons, have narcotic properties which increase with the number of carbons within the ring system. With introduction of side chains into these ring systems the toxicity increases with the length of the side chain, which increase is especially marked in the cyclo-hexane series between the methyl and ethyl radicals. As observed with the olefines, introduction of a double bond increases the narcotic and toxic action, and the margin of safety between them appears to be quite small. Like other hydrocarbons, cycloparaffins may cause irritation of the skin and mucous membranes.

In comparison with the aromatic hydrocarbons, the effect of aliphatic hydrocarbons on the blood and blood-forming organs, their fate in the metabolism, and their excretion, has not been adequately studied, and it appears that the saturated and unsaturated cycloparaffins especially may prove interesting in this respect.

2. The *aliphatic alcohols* are formed by the introduction of one hydroxy group into aliphatic hydrocarbons, and this tends to increase the narcotic action, at least with the lower homologues of this series. Only the lower members of this series, from methyl to amyl alcohol, are used as industrial solvents; those with more than five carbons are slightly volatile, and those containing ten carbons and more are solids at normal temperature. With those members of the series which are of industrial interest, both the boiling point and the partition coefficient $\frac{\text{oil}}{\text{water}}$ increase with the molecular weight, and these physico-chemical characteristics are presumably largely responsible for the duration and intensity of their narcotic action.

They are readily absorbed from the mucous membranes of the respiratory and gastro-intestinal tracts, whereas their absorption through the intact skin appears to be limited.

In regard to their fate in the organism, the primary alcohols of the type R OH are oxidized to aldehydes and monocarboxylic acids, whereas secondary alcohols of the type R OH R are oxidized to ketones and carboxylic acids. Methyl and ethyl alcohols are representatives of the former type, and isopropyl alcohol is a representative of the latter type.

As pointed out before, the narcotic action of alcohols increases with the molecular weight, and it is paralleled by an increase of the boiling point and the partition coefficient $\frac{\text{oil}}{\text{water}}$. Alcohols with branched chain (iso) appear to be

less effective than those with straight carbon chains. Whether this should be explained on the basis of physico-chemical properties, or whether it is associated with a different speed of oxidation appears to require further study. In some instances, as is the case with butyl alcohols, the secondary alcohols are more effective than the normal compounds, presumably on account of their greater vapor tension.

Toxicity and narcotic action do not run parallel. The lowest member of this group with the least marked narcotic action, methyl alcohol, is more toxic than the higher homologues, and it appears that the toxicity is in part determined by the metabolites, that is, in this specific case, by the formation of formaldehyde and formic acid. In addition, the toxicity is partly determined by the speed of the oxidation of the alcohols in the organism, partly, as far as the excretion through the lungs is concerned, by their volatility, and partly by the speed of their urinary excretion.

The local irritant action of alcohols depends in part upon their efficiency as fat solvents, in that they remove in this way the protective fat layer of the skin and thus render it more susceptible to thermic and atmospheric influences. With the lower homologues, which have a comparatively high affinity to water, dehydrating effects may also be involved. The precipitant action of alcohols on proteins, which increases from methyl to amyl alcohol and which is probably related to corresponding changes of the surface tension, seems to be another factor in the irritant action. Especially with the higher members of this group the local irritation is followed by analgesia which is especially marked with butyl and amyl alcohol and which may be connected with the above effect.

In regard to the possibility of habituation to alcohol, this depends within certain limits upon the individual constitution, especially on the metabolic rate and on the functioning of the liver which determines the speed of the destruction of the toxic agent.

Of *bivalent alcohols* the lower homologues, especially ethylene glycol, HOCH —CH OH, and its derivatives, are extensively used as solvents for cellulose lacquers. The introduction of a second hydroxy group reduces the narcotic action as compared with that of monovalent alcohols. Ethylene glycol itself has very moderate narcotic and some irritant properties, and, on account of its high boiling point, systemic effects from the inhalation of its vapors have not been observed. With oral administration, however, it will cause severe injury of the kidney which is, in part, due to its fate in the metabolism, resulting in the formation of oxalic acid. This appears to depend on the existence of two carbon compounds, both carbons of which are partially oxidized. Among the ethers of ethylene glycol, diethylene glycol has moderate irritant and narcotic properties, and, when taken by mouth, marked nephrotoxic action which is more marked with the double ether of ethylene glycol, dioxan. On account of its low boiling point the latter may also be dangerous by absorption through the lungs. With the monoalkyl ethers of ethylene glycol, the nephrotoxic action and the irritant action appear to increase from the methyl to the butyl derivative. The narcotic action is not very marked and its trend within the series has not yet been estab-

lished. The diethyl ether is slightly less active than monoethyl glycol. Of the esters, mono- and diacetyl glycol have a distinct nephrotoxic action, being at least partly hydrolysed and oxidised to oxalic acid. Of the mixed ether-esters, methyl glycol acetate has a more marked nephrotoxic action than the ethyl ether, the narcotic and irritant properties being not very marked.

In opposition to ethylene glycol, propylene glycol is comparatively little toxic although the irritant action, especially on mucous membranes, appears somewhat more marked, and its monomethyl ether is said to have only moderate irritant, nephrotoxic and narcotic properties. The toxicity is further reduced with the next higher homologue, butylene glycol, which is nonirritant and of low narcotic and nephrotoxic action, which properties are only moderately increased by esterification with acetic acid.

Of *trivalent alcohols*, only glycerol is of industrial importance. It is largely used as a softening agent. It has some irritant and moderate narcotic and nephrotoxic action, the latter becoming manifest only after the ingestion of fairly large doses. Under certain conditions it may be utilized by the organism and changed to glucose, which effect becomes more marked with the higher homologues, mannite and dulcite which, however, are at present of no great industrial importance.

3. Oxidation of primary alcohols yields *aldehydes*, of which formaldehyde and acetaldehyde are the main representatives. They have narcotic and irritant properties, the former being prevalent with the higher and the latter with the lower homologues, depending partly upon their solubility in water, which is greater with the lower members of this group. The lowest member of this series is mostly used as a disinfectant and in the manufacturing of artificial resins, and its polimer, paraldehyde, is used industrially in mixture with alcohol as a solvent for nitrocellulose. As compared with the monomer, the irritant action of the polymer is considerably reduced, whereas the narcotic action is materially increased, so that it can be used effectively as a sedative and soporific. The irritant action is considerably increased by the introduction of a double bond as in acroleine which is formed by dehydration of glycerol as by heating fat and oils. It has very marked irritant properties, whereas its narcotic action is negligible. Of cyclic aldehydes, only furfural (furfurol) is of industrial importance, being used as solvent for cellulose esters and waxes, resins and caoutchouc, and also in the vulcanization of rubber. It has irritant properties, but on account of its high boiling point the stimulant and depressant effect on the central nervous system is of no industrial toxicological importance.

Condensation of aldehydes with monovalent alcohols yields *acetals*. Of these, only the condensation product of acetaldehyde with ethyl alcohol, diethylacetal, has any industrial importance, being used in the paint and varnish industry, but it can hardly be considered an industrial poison and, although moderately irritant, it has been used therapeutically on account of its narcotic action.

4. Oxidation of secondary alcohols yields *ketones*. These are characterized by a C=O group bound to two alkyl radicals. As in the case of aldehydes, the oxygen is connected with the carbon atom by a double bond, which accounts

for the great activity of these compounds. They are used extensively as solvents for celluloid, cellulose acetate, and nitrocellulose in the lacquer industry. Their vapors cause more or less marked irritation of the mucous membranes, which is, however, less pronounced than with aldehydes, and they have narcotic properties which are more marked than those of the corresponding alcohols. With the simple ketones, such as dimethyl ketone (acetone), methyl-ethyl ketone (butanone), methyl-propyl ketone (propanone-2), methyl-n-butyl ketone (hexanone-2), methyl-n-amyl ketone (heptanone-2), and methyl-n-hexyl ketone (octanone-2), the narcotic action increases with the number of carbons, and the degree of depression is closely paralleled by an increase of their partition coefficient, $\frac{\text{oil}}{\text{water}}$, and of their surface tension. A comparison of the narcotic action of hexanone-2 with that of other ketones with six carbons but of different structure, such as methyl-iso-butyl ketone (hexone), methyl-iso-butyl ketone (mesityl oxide), diacetyl ethane, cyclo-hexanone and methyl cyclohexanone, indicates that those with a branched side chain (hexone) are less effective than those with a straight side chain (hexanone). Comparison of the narcotic action of cyclohexanone and methyl cyclo-hexanone indicates that the narcotic action is increased by the introduction of an aliphatic radical, as is also the case with the introduction of a double bond (mesityloxide). The presence of a second ketone group, as in diacetyl ethane, reduces the narcotic action.

5. Upon further oxidation, primary alcohols yield *monobasic acids* of the general formula RCOOH. The lower homologues of this group, such as formic and acetic acid, are used as mordants in the textile industry. They have no narcotic properties, but their vapors produce irritation of the mucous membranes.

Oxidation of bivalent alcohols yields *dibasic* acids of the general formula HOOC R—R COOH. Of these only oxalic acid is of greater industrial importance, being extensively used as a bleaching agent. Locally it acts as an irritant, and following its absorption it has marked systemic effects which are closely affiliated with its ability to form insoluble calcium salts.

6. Complete oxidation of aliphatic hydrocarbons and of all carbonaceous material yields *carbon dioxide*. This is extensively used as a refrigerant. When solid, its contact with the skin may give rise to burns of various degree, whereas inhalation of the gas causes stimulation and, in high concentrations, paralysis of the respiratory center.

Less complete oxidation yields carbon monoxide. This is a by-product of many industrial processes. Its toxic action is mainly based on its great affinity to hemoglobin, thus preventing the formation of oxyhemoglobin and leading mainly to anoxemia, the sequelae of which vary with the intensity and duration of exposure. Among other aliphatic oxides, *ethylene oxide* should be mentioned. It is used as a fumigant and intermediate in chemical industries. It has narcotic and irritant properties.

7. Condensation of aliphatic acids with alcohol yields *esters*. These are used extensively as solvents in the lacquer and other industries. The lower members of this series and, less readily, the higher homologues are hydrolyzed with the

formation of their constituents, and their toxicity depends partly on their split products. The dangers from their absorption through the lungs and the skin are determined partly by their solubility in water and partly by their volatility.

The formic acid esters, methyl, ethyl, n-butyl and iso-amyl formate, are especially easily hydrolyzed and are, therefore, generally more toxic than the esters of higher aliphatic acids, and their irritant and narcotic action increases from methyl to iso-amyl formate, whereas cyclic formates are said to be less toxic, approaching, in this respect, amyl acetate.

The acetic acid esters, methyl, ethyl, propyl, n-butyl and iso-amyl acetate, have irritant and narcotic properties which increase, with the molecular weight, from the methyl to the amyl compound. Of the cyclic acetic acid esters, cyclohexylacetate is said to be three times more toxic, but three to five times less volatile, than amyl acetate, whereas benzyl acetate is said to be more irritant.

Esters of propionic and butyric acid, although also used as solvents, have evidently not been studied toxicologically, and esters of higher aliphatic acids, such as ethyl and butyl lactate, are said to be little toxic.

In man, exposure to the vapors of esters has been found to cause irritation of the mucous membranes (conjunctivitis and irritation of the upper respiratory tract), their contact with the skin may lead to irritation and secondary infections, and, with some, systemic effects of various natures have also been reported.

8. Condensation of two molecules of alcohol yields *ethers* of the general formula R—O—R which have marked narcotic properties. Of the three lowest members of this group, dimethyl, diethyl and dipropyl ether, only the diethyl ether is of great industrial importance, being used as a solvent in the manufacturing of smokeless powder and artificial silk (Chardonnet process).

9. Introduction of one or more halogen atoms into aliphatic hydrocarbons yields a great series of halogenated hydrocarbons which are extensively used in industries. Substitution of one, two, three, or four hydrogen atoms of methane by chlorine yields *mono, di, tri and tetra-chloro methane*, the two latter being better known as chloroform and carbon tetrachloride. The lowest member of this group is used as a refrigerant and the others play a very important rôle as solvents, especially for fats and oils. The hemolytic, antiseptic and narcotic properties of these compounds increase with their molecular weight and in inverse ratio to their solubility in water. The irritant action on the mucous membranes is much more marked with the lower than with the higher members, and the injurious effect on the liver increases with the number of chlorine atoms. Substitution of bromine for chlorine decreases, as a rule, the narcotic action but increases the toxicity, and the same holds true, as far as is known, to a larger extent for iodine derivatives. On the other hand, the substitution in carbon tetrachloride of one or two chlorine atoms by fluorine, resulting in the formation of monofluorotrichloromethane and difluoro-dichloromethane, both of which are used as refrigerants, reduces the toxicity considerably. The lowest member of this series yields, upon hydrolysis, methyl alcohol, and this explains, in part, the toxicological picture.

Introduction of one or more chlorine atoms into the ethane molecule yields

monochlorethane, dichlorethane, ethylidenechloride, alpha-trichlorethane, beta-tri-chlorethane, tetrachlorethane, penta-chlorethane and hexachlorethane. These are extensively used as solvents in various industries. The antiseptic, narcotic and toxic action increases with the number of chlorine atoms introduced, and comparison of the narcotic action and the toxicity of isomers indicates that this is reduced by an unequal distribution of the chlorine atoms between the two carbon atoms. As in the previous series, replacement in tetrachlorethane of two chlorine atoms by fluorine reduces the toxicity materially, and replacement of the chlorine atom by bromine and iodine, as in ethyl chloride, ethyl bromide and ethyl iodide, decreases the narcotic action and increases the toxicity.

Introduction of one or more chlorine atoms into ethylene yields *vinylchloride, dichlorethylene, trichlorethylene and tetrachlorethylene.* These are also used very extensively as solvents in various industries. As compared with the ethane series, the toxicity of these compounds, especially in regard to the liver, is very materially attenuated, and their narcotic action increases with the number of chlorine atoms in the molecule.

Of chlorinated propane derivatives, *propylchloride and trichlorpropane* are of industrial importance. These appear to be less toxic, both regarding the effect on the liver and the narcotic action, than the corresponding ethane derivatives.

Chlorine derivatives of butane, pentane and their higher homologues are at present of minor industrial importance, and very little is known in regard to their toxicology. Of chlorine derivatives of higher hydrocarbons, only allyl chloride and 2-chloro-butadiene have been studied more closely. Allyl chloride is considered to be one of the most toxic members of this group, having a very strong irritating action, especially on the lungs. Its narcotic action is very weak and its injurious effect on the liver is apparently less severe. The chlorine derivative of butadiene, 2-chlorobutadiene (chloroprene), is the starting material for the synthetic rubber "Neoprene". It causes some irritation, has narcotic properties, and, with sufficiently high doses and prolonged exposure, will cause injury of liver and kidneys.

Introduction of chlorine into alcohol molecules yields chlorinated alcohols. Of these, *ethylene chlorhydrine*, $CH_2Cl—CH_2OH$, and *dichlorhydrine*, $CH_2Cl—CHOH—CH_2Cl$, are used as solvents. They cause irritation of the mucous membranes, the former causing, in addition, nervous injuries and fatty degeneration of the liver, heart and kidneys, and the latter affecting also the circulation. Trichlorisobutyl alcohol and tribromoethyl alcohol (avertin) have marked narcotic properties but are not important from the industrial point of view.

Of the halogenated aldehydes, only *chloralhydrate* should be mentioned. This has marked sedative and hypnotic properties being, however, easily injurious to the heart.

Of the halogenated ketones, *chloroacetone, bromoacetone, brom-methyl-ethyl-ketone, dibrom-methyl-ethylketone and iodo-acetone* should be mentioned. They are, at present, of little industrial importance but have been used, on account of their very marked irritant action, especially on the eyes, in chemical gas warfare.

Of the chlorinated esters, *methyl sulfuryl chloride and ethyl sulfuryl chloride* are

used in the chemical industries. They cause severe irritation of the eyes and the respiratory tract and, in addition, systemic effects. Others, as *methyl-chloro-formate, chlormethyl-chloro-formate, dichlormethyl-chloro-formate, trichlor-methyl-chloro-formate, methyl-bromoacetate and ethyl-iodoacetate,* cause severe irritation of all mucous membranes and have been used, for this reason, in chemical gas warfare.

Of chlorinated ethers, only *β,β',-dichlorethyl ether,* $C_2H_4Cl—O—C_2H_4Cl$, is of some industrial importance, being used as solvent in the textile industries. It has marked irritant and some narcotic properties, being also injurious to liver and kidneys.

10. Introduction of an amino group into aliphatic hydrocarbons yields *aliphatic amines,* and, depending upon the number of alkyl radicals introduced into the ammonia molecule, mono-, di- and tri-alkyl amines may be distinguished. As an increasing number of alkyl radicals is introduced the original character of ammonia is attenuated. There exists little information regarding their toxicologic action, and they are, at present, of limited industrial importance.

The same holds true for *amino alcohols* of the type NH_2 R OH. Of these, triethanolamine, $N(C_2H_4OH)_3$, is being used as solvent and detergent. On account of its high boiling point it offers very little risk in regard to toxic effects from inhalation, but absorption through the skin appears to be possible, and this may result in injury of liver and kidneys.

11. Introduction of one or more nitro groups into aliphatic hydrocarbons yields *nitroparaffins.* The lower members of this series, *mono-nitro-methane, -ethane, -propane and -butane,* cause local irritation and injury of the liver, these effects increasing with the molecular weight. *Tri-nitro-methane* may be formed as a by-product during nitration of toluene. It causes marked irritation and may lead to formation of methemoglobin. Its chlorine derivative, *chloropicrine,* is used in fumigation and has marked irritant properties. Of other chlorinated nitroparaffins, *1-chloro-1-nitromethane and 2-chloro-2-nitropropane* have marked irritant properties but are evidently not more injurious to the liver than the corresponding, unsubstituted, nitro compounds.

Introduction of a nitro group into alcohols, as in *2-nitro-2-methyl-1-propanol, 2-nitro-2-methyl-1,3-propanediol and 2-nitro-2-methylol-1,3-propanediol,* results in irritant materials, and, with the latter, other toxic effects may be attributed to the formation of methyl alcohol in the organism.

12. Esters of aliphatic alcohols with nitrous acid of the general formula R—ONO are called alkyl nitrites. Of these, *ethyl nitrite* is used in the chemical industry and may be formed in the manufacturing of mercury fulminate. It produces vasodilatation and fall of blood pressure and may cause methemoglobin formation. The higher homologue, *isoamyl nitrite,* is used in chemical industries and as a therapeutic agent on account of its vasodilator and pressor action; in larger doses it is liable to cause central stimulation and methemoglobin formation.

Representatives of *aliphatic esters of nitric acid* are *tri-nitroglycerol and di-nitro ethylene glycol.* These are extensively used in the explosives industry. Their effect closely resembles that of alkyl nitrites because they are partly hydrolyzed

and reduced in the organism with the formation of nitrites. They cause vasodilatation, fall of blood pressure and, in large doses, central stimulation and methemoglobin formation. Nitro derivatives of higher polyvalent alcohols, such as *erythritol tetranitrate*, have a similar but somewhat delayed action.

13. Aliphatic *nitriles* are characterized by the group —C≡N. The lowest member of this series is formyl nitrile or *hydrocyanic acid*. This is used as a fumigant and may be encountered in the mining, chemical and other industries. It paralyses the cellular respiration, and, in sufficiently high concentrations, may be rapidly fatal. The corresponding compound in the dibasic series is dicyan. This is more irritant but less toxic than hydrocyanic acid. The higher homologues, such as acetonitrile and propionitrile resemble, qualitatively, hydrocyanic acid but are less toxic. The halogene derivatives of hydrocyanic acid, cyanchloride and cyanbromide, have marked irritant properties, and, in addition, have a depressant effect on the cellular respiration similar to that of hydrocyanic acid. They are used with other fumigants as warning agents and may be encountered in chemical industries.

Isonitriles of the general formula C≡NH are used in the chemical industry, and their action is similar to that of the nitriles.

Thiocyanates or rhodanates of the general formula RSCN are used as insecticides. The lower homologues split off hydrocyanic acid but become more stable with increasing molecular weight, so that with dodecyl and lauryl sulfocyanate this effect is absent. However, with sufficiently high doses and with sufficient duration of exposure, the latter may cause degenerative changes of the liver and of other organs.

14. Regarding *aliphatic sulfur derivatives*, carbon disulfide is, by far, the most important, being used as a fumigant and also in the rubber, rayon and various chemical industries. In large doses it acts as a narcotic agent, whereas in lower concentrations it acts mainly as a nerve poison for the peripheral and central nervous system, leading to peripheral neuritis and psychic disturbances.

Replacement of the oxygen atom in alcohols by sulfur leads to *mercaptanes* of the general formula RSH. They are stenches and are used as intermediates in chemical industries. Ethyl mercaptane causes primary stimulation and, later, paralysis of the respiration similar to that produced by hydrogen sulfide. Chlorinated mercaptane, perchlormercaptane, CCl_3—SCl, has marked irritant properties.

Sulfurated ethers of the type RSR may be encountered in the chemical industries. They may cause paralysis of the central nervous system. Of great practical importance is the chlorine derivative, dichlordiethylsulfide, mustard gas, which is used in chemical industries and in gas warfare. It has marked irritant properties, causes vesication, and acts as a cell and nerve poison.

B. THE AROMATIC HYDROCARBONS AND THEIR DERIVATIVES. 1. The *aromatic hydrocarbons*, the lowest homologues of which are benzene, toluene and xylene, are used extensively as solvents in lacquers and spray paints and as starting materials in the manufacture of dyes and in the explosives industries. In judging the toxicity of these materials one has to distinguish between acute and

chronic toxicity and the former may vary according to the form of absorption. The aromatic hydrocarbons generally produce a progressive depression of the central nervous system which, especially with toluene, may later on be associated with convulsions. The reflex excitability usually persists until shortly before death which results from respiratory paralysis. The narcotic and irritant action of toluene and xylene is greater than that of benzene, whereas with continued exposure to lower concentrations the latter is by far the more dangerous on account of its hematotoxic action. In addition to the effect on the central nervous system they cause peripheral vasodilatation. Benzene and those of its derivatives with substitution of two hydrogen atoms in para position (p-xylene, p-methyl-ethyl benzene, p-methyl-propyl benzene and p-diethyl benzene) cause tremors of the body. These gradually pass into twitchings which may persist until the beginning of the paralytic stage. Hydrocarbons with a branched side chain (isopropyl benzene) are generally less toxic than those with a straight side chain (propyl benzene) and the introduction of two or three groups usually produces less toxic substances than that of a single group with an equal number of hydrocarbons. Whereas the acute toxicity of aromatic hydrocarbons has been studied quite extensively, the effect of continued exposure of the higher homologues, especially on the blood picture and on the skin, needs further study. The available information indicates that the hematotoxic action of benzene, resulting in leucopenia and anemia, is considerably attenuated by substitution of hydrogen atoms, as in toluene and xylene. The reason for the outstanding hematotoxic action of benzene, as compared with its homologues, is probably its different fate in the organism, in that it is oxidized in the organism to phenol, pyrocatechol and hydroquinone, whereas the higher homologues are usually oxidized in the side chain with the formation of carboxylic acids. The intensity of the oxidation and the amount of hydroxy derivatives, especially of polyphenols, formed in the liver, may vary with the metabolism. This may explain the variations in susceptibility of different individuals. Such studies may promote considerably the understanding of the mechanism of the toxic action of benzene and its homologues.

2. Introduction of one hydroxy group into the benzene ring with the formation of *phenol* increases the convulsant and decreases the narcotic actions. Phenol is used extensively as an antiseptic and as a starting material in the chemical industry, and it forms one constituent of phenol-formaldehyde plastics. The ingestion of sufficiently large doses of phenol may lead to corrosion of the upper digestive tract and result in convulsions of the spinal type and collapse. Continued exposure to lower concentrations may lead to nervous and nutritional disturbances and injury to the kidney. Contact with the skin will cause irritation and, when continued for a sufficient length of time, may cause necrosis.

As in the case of olefinic hydrocarbons the toxicity of phenol is decreased by hydrogenation, hexahydrophenol (cyclohexanol) being less toxic than phenol. The same holds true for the higher homologue (methyl cyclo-hexanol) which, as well as the former, is used as a solvent for fats, oils, waxes, rubber, etc.

The toxicity of phenol is also reduced by closing the hydroxy group by etheri-

fication with alkyl groups. The methyl ether of phenol (anisol) and its ethyl ether (phenetol), both of which are used in the chemical and explosives industries, are considerably less toxic than phenol and do not cause convulsions.

A similar effect is produced by the introduction of alkyl groups into the ring of phenol. Introduction of one methyl group yields cresol (methyl-hydroxy benzene) and, depending upon the position of these two groups to each other, ortho, meta and para cresol may be formed. These are used as antiseptics and wood preservatives, and also in the chemical industries. They are less toxic than phenol in respect to their systemic action, although their local action is of the same order. They all depress the central nervous system and differ among themselves in that the ortho compound is generally the most toxic and the meta compound the least toxic, the para compound being intermediate. It has been stated that continued exposure to vapors of cresol may cause changes of the blood picture which are similar to but much less severe than those observed with benzene.

Among the esters of phenol and its homologues, the triphenyl and tri-ortho-cresyl phosphoric acid esters are used as plasticizers and solvents for various resins and nitrocellulose. Whereas the former is not very toxic, the ingestion of tri-ortho-cresyl phosphate may lead to peripheral neuritis. The inhalation of its vapors may cause some irritation but nervous injury from this form of administration has not been reported.

Halogen derivatives of phenol are considered to be less toxic than phenol in regard to their convulsant action, but they may cause more or less severe irritation of the skin. Pentachlorphenol is of industrial importance, being used as a preservative for wood. Although administration of sufficiently high doses may cause toxic symptoms, such as accelerated respiration, fever, hyperglycemia, glucosuria, motor weakness and collapse, and the possibility of toxic effects from prolonged contact cannot be excluded, it has evidently not given rise to industrial poisoning.

Introduction of an amino group into the phenol molecule yields *amino-phenols* which are intermediates in various chemical industries. The three isomers, para-, ortho- and meta-aminophenol are considerably less toxic than aniline, and the phenol action is also considerably attenuated. Whereas the para compound very readily forms methemoglobin, this is less marked with the ortho and, especially, with the meta compound. The toxic action is somewhat reduced by replacing one hydrogen atom of the amino group by an acetyl radical (acetyl-aminophenol) or by alkyl groups, as in p-methyl-aminophenol and o-methyl-aminophenol which are used as developers in photography and which may give rise to dermatitis. Whether or not this is a direct action or is due to quinone formation does not appear to be settled. The methemoglobinemic action is abolished by substitution of the second hydrogen atom by an ethyl radical (ethyl-acetyl-p-aminophenol).

The toxicity of aminophenol is also reduced by closure of the hydroxy group by etherification, the ethyl ether, phenetidine, being used as analgetic and antipyretic. Of the corresponding acetyl derivative, phenacetine, which has less

marked side actions than acetanilide, a number of homologues have been prepared and studied. Of these the methyl ether is more and the propyl, butyl and amyl ethers are increasingly less toxic so that it appears that this is a function of the stability of the ether binding. Closure of the hydroxy group of acetyl-p-aminophenol by esterification with an acid, as in diacetyl aminophenol, appears to yield less stable and, hence, more toxic compounds.

Introduction of one or more nitro groups into the phenol molecule yields mono-, di- and tri-nitrophenols which play an important rôle in the explosives industry and which are also encountered as intermediates in the chemical industry and sometimes in other industries.

Of the three mono-nitrophenols the para compound is said to be the most and the ortho derivative the least toxic. They cause stimulation and depression of the central nervous system and are methemoglobin formers.

Of the six isomeric dinitrophenols the 1,2,4-dinitrophenol is most commonly used. The 1,2,4-, 1,2,5- and 1,2,6-dinitrophenols are said to be more toxic than the 1,3,5-, 1,3,4- and 1,3,2-derivatives. These substances increase the oxygen metabolism and raise the body temperature, which effect is, at least to some extent, bound to the presence of one nitro group in para position to the hydroxy group. In addition, they may cause stimulation and, later, depression of the central nervous system, visual disturbances (cataract), injury of liver and kidneys, blood changes (agranulocytosis), and irritation of the skin resulting in dermatitis. Whereas one of the most active hyperthermic agents, 1,2,4-dinitrophenol, produces no methemoglobinemia, the less toxic compounds, 1,3,2- and 1,3,6-dinitrophenol, are methemoglobin formers, so it appears that the hyperthermic and methemoglobinemic actions are linked to different parts of the molecule and are possibly caused by different metabolites. Of the alkyl derivatives of dinitrophenol the 4,6-dinitrocresol and 3,5-dinitrocresol have qualitatively and quantitatively an effect very similar to that of 1,2,4-dinitrophenol, the latter of the two being, however, somewhat less toxic than the former.

Introduction of a third nitro group into the phenol molecule yields trinitrophenol. Of the four isomers, 2,4,6-trinitrophenol (picric acid) is the most important, being extensively used in the explosives and dye-stuffs industries. In contrast to the lower nitration products of phenol, with this compound the irritant action on the skin and mucous membranes is the predominant toxic effect. This may lead to dermatitis, conjunctivitis and bronchitis. With heavy exposure it may lead to nervous and gastro-intestinal disturbances, but it does not produce methemoglobinemia. The decrease of the methemoglobinemic action, with subsequent nitration, and also the decrease of the systemic toxicity, is a phenomenon which will also be observed with other substitutions of inorganic radicals in aromatic hydrocarbons.

Introduction of a second hydroxy group into benzene yields *diphenols*, the ortho compound being known as pyrocatechol, the meta compound as resorcinol, and the para compound as hydroquinone. They are used in photography, in the rubber iudustry, and in the chemical industries. Systemically they cause convulsions of the spinal type, hydroquinone being the least and pyrocatechol

the most effective in this respect. In addition they cause methemoglobinemia, leucopenia and anemia which were also observed in benzene poisoning. Their contact with the skin, especially hydroquinone, may lead to eczema. Some of these effects are evidently affiliated with their ability to form quinones, affecting in this way other oxidation-reduction potentials in the organism. Etherification of one hydroxy group by alkyl radicals reduces the toxicity of diphenols, the mono-methyl ether of pyrocatechol (guajacol) being more toxic than the corresponding hydroquinone derivative, and it appears that with increasing molecular weight the toxic action of diphenol mono ethers decreases, whereas the local action increases.

Introduction of a third hydroxy group into the benzene molecule yields triphenol, and, according to the relation of these to each other, three isomers may be formed. Two of these, 1,2,3-trihydroxy benzene (pyrogallol) and 1,2,5-trihydroxy benzene (phloroglucinol), are used in various industries. Their depressant and convulsant actions are considerably decreased as compared with those of benzene and phenol. The 1,2,3 compound is said to be twenty times more toxic than the 1,2,5 derivative which is practically inactive. The former may cause irritation and erythema of the skin, and when absorbed may cause methemoglobinemia, hemolysis and nephritis with hematuria.

3. The *aldehydes* of the aromatic series, such as benzaldehyde, protucatechu aldehyde and vanilline, are of limited industrial importance. The latter is said to cause headache, vertigo and somnolence during the day; nervousness and insomnia during the night. They are readily oxidized in the organism, resulting in practically inert carboxylic acids.

4. The *aromatic carboxylic acids*, such as benzoic acid and its derivatives, amino and o-hydroxy benzoic acid (salicylic acid), as well as the dicarboxylic acid, phthalic acid, are used in the chemical industry and are of very limited toxicity. Only the anhydride of the latter, phthalic acid anhydride, is of some toxicological importance. It causes irritation of the eyes and the respiratory tract on account of its dehydrating action. Several esters of phthalic acid, dimethyl phthalate, diethyl phthalate and dibutyl phthalate, have recently gained some industrial importance as plasticizers for nitrocellulose and acetyl cellulose. On account of their high boiling point the danger of toxic effects from the inhalation of their vapors seems to be remote. Exposure to a mist of these materials results in irritation of the mucous membranes of the eyes and the respiratory tract. The use of diethyl phthalate as a denaturing agent for alcohol has given rise to irritation of the skin, characterized by itching, eczema and paresthesias of the fingers. It may also have some effect on the blood and blood-forming organs, and may cause injury to the liver and kidneys. However, the mechanism of this action appears to need further study.

5. Of the halogenated benzene derivatives, only the chlorine compounds, mono-chloro-benzene and di-chloro-benzene, are of industrial importance. These are used as solvents for acetyl cellulose, resins and fats, as cleaning agents for metals and as intermediates in the chemical industries. Mono-chloro-benzene causes moderate irritation of the skin and mucous membranes. It

depresses the central nervous system but is said to be less toxic than benzene. Di-chloro-benzene is also used as an insecticide and fumigant and, technically, usually as a mixture of the two isomers, ortho- and para-dichloro-benzene, the latter being considered more toxic. Their depressant effect on the central nervous system appears to be more pronounced than that of the mono derivative. The effect of these substances on the blood pigment, the blood, and the blood-forming organs has not been studied, and a study of their effect on the hemopoietic system, especially in relation to their fate in the metabolism in comparison with that of benzene, and their hepatotoxic action may be of considerable interest and of practical importance.

Introduction of halogens in an aliphatic side chain, as in that of toluene, yielding benzyl chloride, benzyl bromide and benzyl iodide, results in chemicals of extremely irritant character which led to their use as war gases.

The chlorination product of diphenyl, diphenyl chloride, is used in synthetic waxes. It causes irritation of the skin and injury to the liver, being more toxic in this respect than the chlorinated naphthalenes.

6. Introduction of an amino group into the benzene ring yields aminobenzene (aniline) which is used extensively in the chemical and explosives industries, in dyeing, and in the manufacture of rubber articles. It is a blood and nerve poison, causing methemoglobin formation, primary excitation followed by depression of the central nervous system, and, finally, loss of consciousness. With prolonged exposure, anemia, circulatory irregularities, disturbances of the gastro-intestinal tract, injury to the kidneys and nervous disturbances may be observed. The methemoglobin formation is not caused by aniline itself but is presumably due to its metabolite, p-aminophenol, and its oxidation product, quinone-imine.

Introduction of alkyl and aryl radicals into the amino group, as in methyl aniline, dimethyl aniline, diethyl aniline, diphenyl aniline and benzyl aniline, yields compounds which are used as intermediates in the chemical industries. The alkyl derivatives differ only quantitatively from aniline, the fundamental toxicological character of the mother substance being unaffected. With the aryl derivatives, on the other hand, not only is the toxicity reduced but the character is also changed, the methemoglobin formation being missing.

Introduction of formic acid and acetic acid radicals into the amino group of aniline yields formanilide and acetanilide which are used in the chemical and rubber industries, the latter being also used as an analgesic and antipyrectic in medicine. Their action is, qualitatively, very similar to that of aniline but much less abrupt, and both are methemoglobin formers.

Substitution of the second hydrogen of the amino group of aniline by a methyl group yields methyl formanilide and methyl acetanilide, both of which are more toxic but less effective as antipyretics. Whereas the methemoglobinemic action of the latter is less marked, as compared with acetanilide, this is not the case with methyl formanilide. This may indicate a greater stability of methyl acetanilide.

Introduction of one chlorine atom into aniline yields monochloroaniline, in which the methemoglobin formation is slightly attenuated and the meta and

ortho compounds are apparently slightly less toxic than the para compound. Introduction of a second and third chlorine atom, as in di- and tri-chloroaniline, decreases only slightly the methemoglobinemic action. This is also the case with mono-, di- and tri-chloroacetanilide.

Introduction of one nitro group into aniline yields o-, m- and p-nitroaniline which are intermediates in the explosives industry. Their toxic action does not differ materially from that of aniline. Further nitration, resulting in tetranitroaniline, evidently reduces the toxicity, as is also observed with tri-nitrophenylmethyl-nitramine, tetryl, but both of these and also dimethyl amino-p-nitrosoaniline cause marked irritation of the skin, leading to papular eruptions and eczema.

Introduction of a second amino group into the benzene ring yields diamino benzene, phenylene diamine. Of the three isomeric compounds, para-phenylene diamine is used as intermediate in the dye industry and forms the main constituent of "Ursol" dyes which are used for dyeing hair and fur. Its methemoglobinemic action is less marked than with aniline, but it causes edema, especially around the neck, irritation of the skin, asthma, and gastro-intestinal disturbances. Large doses may cause convulsions of the spinal type. The methemoglobinemic, edemagenic and irritant actions on the skin have been credited to its oxidation product, quinone diimine. This, in opposition to quinoneimine, the final metabolite of aniline which represents a redoxy system, is readily oxidized further, and this may explain the less marked methemoglobinemic action. The meta compound is less toxic than p-phenylene diamine and the ortho compound is still less toxic, which may indicate that they have a different fate in the organism. Whereas substitution of two hydrogen atoms of one amino group, resulting in dimethyl-p-phenylene diamine, increases the toxicity considerably, the diethyl derivative is said to be about as toxic as the mother substance, but both materials are more readily absorbed through the skin.

Introduction of one amino group into toluene yields amino toluene or toluidine which is used as an intermediate in the dyestuff industry. It exists as ortho-, meta- and para-toluidine. The toxic action of the ortho compound resembles closely that of aniline, the acetyl derivative is also a methemoglobin former, but the antipyretic and analgetic actions are less marked than those of acetanilide. Whereas meta-toluidine is more toxic than ortho-toluidine, m-acet-toluidine does not form methemoglobin, and the same holds true for p-acet-toluidine although p-toluidine itself is the most toxic of the three isomers. The reason for this difference in their behavior may be their different fate in the organism, the ortho compound being largely oxidized to a phenol and the para compound to p-aminobenzoic acid.

The three monochlor-toluidines, 5-chloro-2-toluidine, 4-chloro-2-toluidine and 6-chloro-2-toluidine, are intermediates in the dyestuff industry. Toxicologically they closely resemble toluidine and aniline. With the 5,2 compound, irritation, congestion and hemorrhages of the bladder were observed.

Of the six isomeric diamino toluenes, only 2,4-diamino toluene has been studied extensively. Its irritant action on the skin is similar to that produced

by p-phenylene diamine. It destroys the blood cells and causes severe injury to the liver. The mechanism of both actions has aroused much interest and it appears to be established that the toluylene diamine icterus is not exclusively of hematogenic origin.

Amino xylene, which is used as an intermediate in the dyestuff industry, closely resembles aniline and toluidine in its action. Introduction of two amino groups into diphenyl yields diamino diphenyl. The 4,4′ compound, benzidine, is mainly important as an intermediate in the dyestuff industry. Its action resembles that of other aromatic amines, but it is less liable than most of these to form methemoglobin. However, it may cause anemia and liver and renal injury and it has been credited with the formation of bladder tumors although the latter has not been demonstrated experimentally.

7. Introduction of one nitro group into the benzene ring yields nitrobenzene which is an intermediate in the manufacture of aniline, aniline dyestuffs and explosives. It is also occasionally used in the manufacture of shoe dyes and as a flavoring agent and cheap perfume. Acute poisoning from its ingestion or from inhalation of high concentrations may cause loss of consciousness and paralysis. Continued exposure to lower concentrations may result in blood changes, cyanosis, and disturbances of the nervous functions and of the gastro-intestinal tract with its sequelae. The methemoglobin formation sets in only after a latent period of several hours, presumably because it takes some time to form the metabolites, nitrosophenol and quinoneimine, which are responsible for this effect. The nitrobenzene anemia appears to be primarily due to a destruction of the red blood cells, whereas the effect on the white blood cells is much less conspicuous than with benzene.

Introduction of one chlorine atom into nitrobenzene yields mono-chloro-nitrobenzene which is used in the explosives industry. Qualitatively it resembles nitrobenzene in its toxic action but quantitatively it is considered more dangerous.

Introduction of a second nitro group into nitrobenzene yields dinitrobenzene. Of the three isomeric compounds the meta derivative is the most important, being an important intermediate in the dyestuff industry and an explosive. Exposure to this material may cause anemia, cyanosis, nervous disturbances and injury to the liver and the kidneys. Its methemoglobinemic action is less marked than that of mono-nitrobenzene and, like the latter, it affects mainly the red blood cells and to a lesser extent the white blood cells. Its chlorination product, mono-chloro-dinitrobenzene, is also used as an explosive and its systemic action is very similar to that of dinitrobenzene. It causes, however, very marked irritation and is considered more dangerous than the latter. This irritant effect is very severe with the higher chlorination product, dichloro-dinitrobenzene, with which contact with the skin may result in erythema, edema and necrosis, whereas little is known in regard to its systemic effect.

Introduction of a third nitro group into dinitrobenzene yields trinitrobenzene which has a toxicological action similar to that of dinitrobenzene. It is said to form methemoglobin but it does not appear to be established whether or not

there are quantitative differences between the di and tri nitration product in this respect.

Introduction of a nitro group into the toluene molecule yields mono-nitrotoluene which is an intermediate in the dyestuff and explosives industries. Toxicologically its action is similar to that of nitrobenzene, but whereas the ortho compound is said to be about as toxic as the latter, the para compound is considerably less toxic.

Of the six isomers of dinitrotoluene the 2,4-dinitro compound is the most important, being used in the explosives industry. The pure compound is said to be less toxic than dinitrobenzene, usually causing only general complaints about headache, fatigue, dizziness, moderate anemia and cyanosis.

Of the five isomers of the triple nitration product of toluene, 2,4,6-trinitrotoluene is the most important, being widely used as a high explosive. The literature on the toxicity of the technical product is highly controversial and it appears that the impure material may be considerably more toxic as compared with the relatively low toxicity of the pure compound. Persons handling trinitrotoluene frequently show yellow discoloration of skin and hair, and they suffer from irritation of the mucous membranes and the skin which may develop into various types of dermatitis. Exposure to trinitrotoluene may result in nervous disturbances of general nature or in neuritides, irregularities of the circulation, disturbances of the gastro-intestinal tract, atrophy of the liver, injury to the kidneys, anemia and cyanosis. The two latter may be the only and the most conspicuous phenomena and may be the cause of the icterus. The anemia is mainly characterized by a destruction of the red blood cells, whereas the effect on the white blood cells may be characterized by leucopenia and leucocytosis but is most commonly characterized by a relative lymphocytosis. The cyanosis appears to be due partly to the formation of methemoglobin and partly to the formation of pigments formed from trinitrotoluene in the organism.

The nitration products of xylene appear to be less toxic than those of toluene and benzene, mono-nitroxylene being considerably less toxic than nitrobenzene, dinitroxylene less than dinitrobenzene and dinitrotoluene, and trinitro-, and especially tetranitroxylene being only very little toxic. It appears, therefore, that the presence of one methyl group reduces the toxicity of the aromatic nitro compounds, and the presence of two methyl groups reduces it further.

8. Introduction of a hydrazine radical into the benzene molecule yields phenylhydrazine which is used in laboratories and in the chemical industries. This material should be handled very carefully as contact with the skin causes erythema, itching, pustulous and papulous eruptions and edema. Continued exposure may lead to sensitization, resulting in allergy characterized by gastro-intestinal disturbances and attacks of asthma. Larger doses may cause irritation of the kidneys, anemia and cyanosis. The destruction of red blood cells is said to be more marked than was observed with other aromatic amino compounds, as, for instance, diaminotoluene, and for this reason phenylhydrazine has been used therapeutically in the treatment of polycythemia. The cyanosis is partly due to the formation of a pigment from phenylhydrazine and partly caused by formation of a methemoglobinlike substance which evidently is not identical

with ordinary methemoglobin. The higher homologue, toluylhydrazine, is said to be less destructive to red blood cells, and the meta derivative, but not the ortho and para compounds, causes facial edema, as observed with paraphenylenediamine.

C. NAPHTHALENE AND ITS DERIVATIVES. Naphthalene, a hydrocarbon consisting of a double benzene ring, is handled in various chemical industries and especially in the dyestuff industries, and it is also used as an insecticide. Being solid and not very volatile, it has comparatively little toxic effect from the point of view of industrial exposure. Inhalation of low concentrations may cause headache, somnolence, and loss of appetite. With more severe exposure these effects become more marked and there may be, in addition, some injurious effects on the kidneys, resulting in albuminuria and hematinuria. Its contact with the skin causes irritation, resulting in itching, erythema and eczema. It is also irritant to the mucous membranes of the respiratory tract and the eyes. On the latter it may cause punctate turbidities of the cornea, cataract and, occasionally, optic neuritis. Its destructive effect on the blood is not marked and certainly not comparable to that of benzene.

Its hydrogenation products, tetrahydro- and decahydronaphthalene, tetraline and decalene, are liquids of high boiling point which are used as solvents for fats and waxes. They are only slightly toxic and their toxic character is similar to that of naphthalene.

1. Introduction of one *hydroxy group* into the naphthalene molecule yields two isomeric naphthols which have qualitatively the same toxic effects. They are important intermediates in the dyestuff industry. They are readily absorbed through the skin and cause irritation of the skin and the mucous membranes of the respiratory tract and the eyes, causing conjunctivitis, temporary turbidity of the cornea and, after absorption of larger amounts, cataract and injury of the retina. Absorption of sufficient quantities may lead to irritation of the kidneys, resulting in the excretion of albumen, blood and hematin.

2. Chlorination of naphthalene leads to tri-, penta- and hexachloro-naphthalene which are constituents of synthetic waxes. Their toxicity increases with the amount of chlorine introduced and they cause irritation of the skin, characterized by itching, pruritus, erythema, vesicular-edematous dermatitis, and chloracne. Whereas trichloronaphthalene is comparatively innocuous in low concentrations, penta- and hexachloronaphthalene are much more toxic and may cause severe injury to the liver.

3. Introduction of an *amino group* into the naphthalene molecule yields alpha and beta naphthylamine which are important intermediates in the dyestuff industry. The alpha compound is said to be less toxic than the beta compound, especially in regard to the production of bladder tumors which are presumably caused by some metabolite of the latter and which are frequently preceded by strangury and hemoglobinuria. Of the hydrogenation products of naphthylamine the ac-tetrahydronaphthylamine is of interest because it increases the body temperature and the oxygen metabolism. In sufficiently large doses it may cause nausea, vomiting, headache and impaired respiration.

The diamino compound of naphthalene is an intermediate in the dyestuff

industry. It is said to be only slightly toxic and, especially, to be free of the edemagenic action observed with p-phenylene diamine.

4. The *nitration* products of naphthalene, mono- and dinitronaphthalene, both of which are intermediates in the dyestuff industry, are comparatively only slightly toxic, only the absorption of large amounts causing irritation of the kidney. Their fumes are irritant to mucous membranes and may occasionally lead to injury of the cornea.

The double nitration product of naphthol, Martius yellow, has caused no severe poisoning in humans. In animals, comparatively large doses cause irritation of the gastro-intestinal tract and the kidneys, and fever, but no marked effect on the blood and the blood-forming organs.

D. Other organic compounds. Of the heterocyclic organic compounds, pyridine is used as a solvent in chemical industries and as a denaturing agent for alcohol. The latter use is said to have caused irritation of the skin and eczema. Its vapors are irritant to the mucous membranes, especially those of the eyes, and high concentrations may cause headache, fatigue, nausea, loss of appetite, diarrhea, general nervous disturbances, neuritides and loss of consciousness.

Thiophene, the corresponding sulfur compound, is one of the contaminants of crude benzene. Its action is similar to but not quite so marked as that of benzene, especially in regard to its effect on the blood and the blood-forming organs.

None of the polycyclic heterocyclic compounds is of great industrial toxicological importance. Quinoline, which is a starting material in the dyestuff industry, causes in high concentrations some depression of the central nervous system, and acridine, the mother substance of the various acridine derivatives, causes irritation of the skin and the mucous membranes, causing sneezing, coughing and conjunctivitis.

A review such as that presented in the foregoing has necessarily to be restricted to an outline of the toxicological action. In many instances only those substances which are either very toxic or important as therapeutic agents have been studied. The understanding of the mechanism of action of many of these chemicals would be much improved if the outstanding toxicological characteristics could be followed in chemically related compounds, as has been done in a few instances, and if their fate in the metabolism could be studied more closely, especially with regard to their chemical constitution. For the evaluation of their potential dangers with industrial exposure, continued exposure experiments with doses or concentrations similar to those which might be encountered in industry should be studied more extensively, special emphasis being placed on possible nervous and blood changes, and pathological changes of the metabolism and vital organs.

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CHEMOTHERAPY OF AVIAN MALARIA

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The investigation of drugs for their antimalarial action is to a large extent limited to avian infections. Experimental animals for investigations of the chemotherapy of malaria are restricted to various species of birds and monkeys. For a number of reasons, large scale use of the latter is impractical. The present review is confined to the chemotherapeutic aspects of avian malaria: no attempt has been made to include other studies on avian malaria which have contributed to our knowledge of the disease.

METHODS. The canary infected with *Plasmodium relictum* has been the experimental animal most commonly used. However, several other species of *Plasmodium* have been utilized in the canary. Russian investigators have used *P. relictum* infections in small birds other than the canary. Recently, chemotherapeutic studies have been made on infections of *P. gallinaceum* in the chicken, *P. lophurae* in the chicken and duck, and *P. relictum* in the pigeon. *Haemoproteus orizivorae* (not a malarial parasite), a naturally occurring infection in the Java sparrow, has been used: only gametocytes occur in the blood, and schizonts are restricted to the endothelium of the internal organs.

Infection has been produced by inoculation of trophozoites (intraperitoneally, intramuscularly or intravenously) or of sporozoites. The latter method has been used only to a limited extent in chemotherapeutic investigations and has been confined to infections of *P. relictum* and *P. cathemerium* in the canary and of *P. gallinaceum* in the chicken. Little attention appears to have been given to the quantitative dosage of parasites in experimental infections. The infection in the young duck produced by the intravenous injection of blood heavily infected with *P. lophurae* offers many advantages over the other infections which have been used for chemotherapeutic studies (Hegner, West, Ray and Dobler, 1941; Coggeshall, 1941; Walker and van Dyke, 1941; Marshall, Litchfield and White, 1942).

Administration of drugs has been by injection (subcutaneously, intramuscularly or intraperitoneally), by stomach tube, and by incorporation in the food. Schedules of doses have varied: the commonest used is that of Roehl (1926), i.e., one dose daily for six days. Very little information is available as to the optimal method of therapy in avian malarial infections. From analogy to what has been established in experimental bacterial chemotherapy, there is reason to believe that frequently repeated doses may be much more effective than a single daily dose although the total amount of drug is the same in both instances. The drug-diet method (Bieter et al., 1940; Litchfield, White and Marshall, 1939), by which a more or less constant blood concentration of drug is maintained, appears to be useful in the treatment of avian malarial infections (Coggeshall, 1941; Walker and van Dyke, 1941; Marshall, Litchfield and White, 1942).

The responses which have been used as criteria of antimalarial activity of

drugs are: 1, reduction of the blood parasite concentration-time curve; 2, significant delay in the incubation period (time of appearance of parasites in the blood), and 3, decrease in the relapse rate. In infections, where mortality of untreated birds is high, survival time or percentage survival may have advantages over the responses mentioned above.

The quantitative methods for comparing the activity of drugs so far published are not entirely satisfactory. Roehl (1926) estimated the activity of a drug in terms of the minimum dose which caused a delay in the appearance of the parasites in the blood. He then determined the maximum tolerated dose of drug for the canary, and compared drugs on the basis of the chemotherapeutic index. Fourneau and co-workers (1931), using the *Haemoproteus* infection of the Java sparrow, determined the fraction of the maximum tolerated dose which just produced a definite decrease in the parasitemia. This was a measurement of effect on the gametocytes of a parasite which is not a member of the genus *Plasmodium*. Buttle, Henry and Trevan (1934) used groups of 6 canaries; one group was left untreated; one group received one dose per day for 6 days of quinine, and other groups received the same doses of different drugs. The mean time before the parasites appeared in the blood was determined for each group. These authors emphasized the importance of using in each experiment a group of birds treated with quinine to serve as a standard of comparison, and attempted to calculate the error of the comparisons.

There are several objections to the above methods. It is questionable whether the chemotherapeutic index of a drug determined for the bird is of any value in assessing the drug for clinical use. Thus, although plasmochin has a much higher chemotherapeutic index than quinine in the bird, it is well known that with plasmochin the margin of safety in the human is too small to allow its extensive clinical use. Differences in absorption, excretion and toxicity of different drugs for the bird as compared to the mammal are almost certain to invalidate the transference of a chemotherapeutic index from one class to the other. Buttle and co-workers (1934), whose quantitative method appears to be the best of the three mentioned above, used the bird solely for assessing the comparative antimalarial value of the drugs, and performed toxicity determinations of the drugs on mice. This procedure of using the bird for assaying the therapeutic effect, and mammals for the toxic effect, appears to be the most satisfactory available at present.

Another very probable source of error in these quantitative methods is the difficulty of obtaining an accurate comparison of drugs on a single daily dosage schedule. Differences in the absorption, excretion, and destruction of two drugs may render the comparison misleading even for the bird. An example of such errors in the comparison of the activity of two drugs on the single daily dosage schedule may be given from data obtained in bacterial chemotherapy. Thus, when diaminodiphenylsulfone and sulfanilamide were tested on a streptococcus infection in mice by the single daily dose method, the former drug was found to be 100 times as active as the latter; when tested on the basis of maintained blood concentrations of the two drugs, their activity ratio was only 3 to 1 (Marshall,

Litchfield and White, 1940). On the basis of experiments on sulfonamide therapy in duck malaria, Marshall, Litchfield and White (1942) suggest that the antimalarial activity of drugs can be assessed quantitatively by determining the blood concentration of each drug which gives the same response. They suggest as the responses to be used parasitized cell counts, survival time or percentage survival as these three responses are highly correlated with each other. Even where it is impossible to determine the blood concentrations of drugs, a comparison of activity, based on a dosage schedule, by which more or less constant blood concentration is maintained, would appear to be less in error than one based on single daily doses.

Just how much the single daily dosage schedule may invalidate conclusions as to the qualitative antimalarial activity of drugs is not known. There are several reports stating that drugs, which showed activity in simian and/or human malaria, were completely inactive in avian malaria (Christophers and Fulton, 1938; Fulton, 1940). Possibly if these drugs were retested with a more efficient dosage schedule some activity would be found. At least, several sulfonamide drugs, which have been reported as inactive in avian malaria when tested by the single daily dose schedule (Coggeshall, 1938; Buttle, 1939; Manwell, Counts and Coulston, 1941; Hegner, West and Dobler, 1941), have been found active when examined by the drug-diet method (Coggeshall, 1941; Walker and van Dyke, 1941; Marshall, Litchfield and White, 1942).

Therapeutic effect of quinine and other drugs. A number of investigators have studied the therapeutic effect of a variety of drugs in avian malarial infections produced by injection of infected blood (trophozoite infection). Kopanaris (1911) found quinine had a curative action but no protective action (when mixed with the inoculum) on *P. praecox* infections in the canary, and that arsphenamine and atoxyl were without effect. Marks (1914) saw no effect on canary malaria from the injection of methylene blue, but a marked effect when the drug was given in the bird's food. The Sergent brothers (1921) carried out the first extensive study of the action of quinine in canary malaria. They differentiated between "curative" and "preventive" treatment, worked out non-toxic doses of quinine, and showed that the drug did not eradicate the infection. They also found that the same amount of quinine given as three doses a day was less toxic and more effective than when given as a single dose. For prevention, they found it useless to start treatment before infection, but necessary for the best result to start on the day of infection. Also, when given every day the drug was more effective than when given every other day or once a week. When quinine was given every day or every other day for 1 to 9 months, the parasites in 1 bird became quinine resistant; in 2, they remained virulent; in 2, they were destroyed; and in 3, they were attenuated in virulence.

Further studies of the effect of quinine or other alkaloids of cinchona bark either in delaying the appearance of the infection or of decreasing an already established infection of canaries have been reported by the Sergents and Catanei (1923, 1924), Sergent and Catanei (1925), Ainley and King (1938), Boyd (1925), Giemsa, Weise and Tropp (1926), Morgenroth, Abraham and Schnitzer (1926),

Kikuth and Tropp (1927), Katahira (1929), Fourneau and co-workers (1930), Goodson, Henry and Macfie (1930), Giemsa and Oesterlin (1933), Buttle, Henry and Trevan (1934), Missiroli (1937), Cohen and King (1938), and Prelog, Stern, Seiwerth and Heimbach-Juhász (1940). Attempts have been made by many of the above workers to assess the comparative therapeutic efficiency of the alkaloids of cinchona bark. Giemsa, Weise and Tropp (1926) compared the action of various quinine derivatives on canary malaria with that on human malaria. Table 1 gives their results.

The naturally occurring *Haemoproteus* infections in the Java sparrow and pigeon (where gametocytes only are present in the blood) were used by the Sergents (1907) and by Collier and Krause (1929) for testing antimalarial drugs. This method has been developed extensively by Fourneau and co-workers (1931). They found quinine to have no effect: stovarsol and methylene blue were quite active. A number of 8-amino quinoline derivatives were tested by

TABLE 1

DRUG	CANARY MALARIA DELAY IN APPEARANCE OF PARASITES OVER CONTROLS	HUMAN MALARIA ACTIVITY
	days	
Cuprein	0–4	0 or +
Quinine	12–13	++
Quinidine	12	++
Chinchonin	0–5	+
Chinpropyrlin	1–4	+ or ++
Hydroquinine	11	++ or ++
Optochin	6–11	+ or ++
Chitenin	0	0
Chitenin-ethylester	5–12	+ or ++
Chinicin	0	0

Fourneau, Tréfouels, Bovet and Benoit (1933); quinine and atabrine were found inactive by Bovet and Demanche (1933). Bovet, Benoit and Altman (1934) found the chemotherapeutic index of 8-diethylamino-undecylamino-6 methoxy-quinoline to be 3 on *Haemoproteus*, but 100 on *Plasmodium* in the canary. The action of drugs on *Haemoproteus* infections has also been studied by Mochkovski (1935), Albricht and Nieuwenhuyse (1937), and Kikuth (1938). Durand and Villain (1939) found sulfanilamide to have no effect on *Haemoproteus* infections in pigeons.

Drugs, other than quinine and its derivatives, have been investigated as to their therapeutic effect in canary malaria. In 1926 Roehl introduced plasmochin (Schulemann, 1932). He found it to be a much more potent drug than quinine, and to have a much higher chemotherapeutic index for *P. relictum* infections in the canary. Hegner and Manwell (1927) confirmed the efficacy of plasmochin in *P. cathemerium* infections of the canary. Other studies on quinoline compounds related to plasmochin are reported by Collier and Warstadt

(1931), Fourneau et al. (1930), Sergent and co-workers (1931), John and Glowasky (1933), Magidson and Strukow (1933), Kritschewski and Sternberg (1933), Tate and Vincent (1933), Sternberg (1934) and Magidson, Madazema and Rubzow (1935). Atabrine, introduced by Kikuth (Kikuth, 1932; Kikuth and Schönhöfer, 1935), acts strongly on canary malaria, but not upon the *Haemoproteus* infection of the finch. Recently, Kikuth (1938) announced a new antimalarial of the quinoline type, "Certuna" (α-dialkylamino-oxyquinolylbutane).

Stovarsol, which is stated to be effective in benign tertian malaria of man, appears to have no action on *P. relictum* infections in the canary (Sergents and Catanei, 1925; Fourneau et al., 1930). Methylene blue, which has some effect on the quartan human infection, possesses only a slight action on *P. relictum* in the canary, but has a marked action on *Haemoproteus* in the Java sparrow (Fourneau et al., 1930; Bovet and Demanche, 1933; Albricht and Nieuwenhuyse, 1937). Mercurochrome, reported to be of some value in benign tertian malaria, appeared to show some effect in *P. cathemerium* infections of the canary (Swezey, 1935). The few amidines which have been tested are reported to be inactive in canary malaria (Easson and Pyman, 1931; Christophers and Fulton, 1938; Fulton, 1940).

Although sulfanilamide was first reported to be of value in the treatment of human malaria, these promising reports have not been confirmed. In both acute and chronic infection with *P. knowlesi* in the monkey, Coggeshall (1938, 1940) has shown that sulfanilamide effects a complete and permanent cure. In *P. relictum*, *P. cathemerium*, and *P. nucleophilum* infections of canaries, neither sulfanilamide nor sulfapyridine were found to have any effect (Coggeshall, 1938; Buttle, 1939; Manwell, Counts and Coulston, 1941): in *P. relictum* infections, a number of sulfanilamide derivatives with side chains resembling that of plasmochin were reported as inactive (Walker, 1940). In contrast to these negative results are the observations that neoprontosil caused complete disappearance of parasites in *P. praecox* (?) infection in Java sparrows (Africa, Dy and Soriana, 1939) and that sulfapyridine had a favorable effect on *P. circumflexum* infections of the canary (Manwell, Counts and Coulston, 1941).

Very few chemotherapeutic studies on infections in avian hosts other than the canary have been reported. Russian workers (Kritschewski and Rubinstein, 1932; Kritschewski and Sternberg, 1933; Mochkovski, 1935) report the use of *P. relictum* in *Acanthis linaria*, *Spinus spinus*, and *Fringilla linaria*. Brumpt, Bovet and Brumpt (1937) tested the effect of drugs on *P. gallinaceum* in the chicken. Coatney and Young (1939) studied the action of colchicine on *P. relictum* infection of pigeons. The activity of quinine and hydroxyethylapocupreine has been reported in ducks infected with *P. lophurae* and in pigeons with *P. relictum* (Hegner, West, Ray and Dobler, 1941; Hegner, West and Dobler, 1941). In *P. lophurae* infections of chickens and ducks several sulfonamides were reported to be entirely without influence on the infection (Coggeshall, 1938; Hegner, West and Dobler, 1941). However, with the use of the drug-diet method of therapy, sulfonamides have been found to be active in this

duck infection (Coggeshall, 1941; Walker and van Dyke, 1941; Marshall, Litchfield and White, 1942).

Action of drugs on different stages of parasite. It has been assumed that there are four stages in the life-cycle of the malarial parasites where a drug may act: 1, on sporozoites; 2, on trophozoites; 3, on gametocytes, and 4, on hypothetical forms responsible for relapse (Schulemann, 1932). All of the chemotherapeutic investigations discussed above have dealt with trophozoite infections (injection of blood of an infected bird into a clean bird) or naturally occurring gametocyte infections of the blood stream. Very few chemotherapeutic studies have utilized sporozoite infections or mosquito transmission of the disease. Manwell (1932, 1934) stated that in trophozoite infections of the canary with various species of *Plasmodium*, treatment with quinine and plasmochin was much more effective when started at the time of infection than if delayed until parasites were present in the blood. Missiroli (1937) also found that quinine was more effective if given during the incubation period than if given when parasites were present in the blood. However, Brumpt, Bovet and Brumpt (1937) stated that with mosquito infections of *P. gallinaceum* in the chicken, quinine, plasmochin and atabrine were curative in the sense that when administered after the parasites had appeared in the blood, they markedly decreased the infection. However, if treatment with these drugs was started at the time of the infection, the incubation period was not prolonged.

Is any drug a prophylactic in the sense of preventing the infection? Russell (1931) stated that plasmochin was an effective prophylactic against *P. cathemerium* trophozoite infection in the canary: Hegner and Manwell (1927) did not find this to be true. On the other hand, Russell (1932) found that plasmochin was not a prophylactic for infections of *P. cathemerium* produced by mosquito bites or by injection of sporozoites. Also, Russell (1934) stated that atabrine did not have a prophylactic action in *P. relictum* infections caused by injection of sporozoites. On the other hand, the Sergents (1922) working with *P. relictum* in the canary found that quinine given immediately after an infection produced by the injection of sporozoites was more effective than in an infection produced by trophozoites. However, if mosquitoes were used, the drug was less effective. Tate and Vincent (1933, 1934) in two very carefully controlled investigations showed that plasmochin and atabrine were very effective in prolonging the incubation period or preventing infection in canaries with *P. relictum* when blood inoculation was used but had no effect on the incubation period when infection was caused by bite of infected mosquitoes. They stated, however, that with the mosquito infection the disease was milder after the administration of atabrine than in the controls. Missiroli (1937) found a definite influence of quinine on a *P. relictum* infection produced by injection of sporozoites in canaries: his data indicated no difference between the effect of quinine in the sporozoite infection and one produced by trophozoites. Godoy and Lacorte (1928) found that plasmochin caused a disappearance of gametocytes from the blood of pigeons infected with *Haemoproteus*, and also acted on sporozoites. They believed that the action of a drug on gametocytes was correlated with its

action on sporozoites. Wampler (1930) found that plasmochin caused first a disintegration of trophozoites of *P. cathemerium* in the canary and later an effect on the gametocytes.

Very few reports of the effects of drugs on the exoerythrocytic stages of avian malarial parasites (Porter and Huff, 1940) have appeared. Neither quinine nor atabrine appeared to have any appreciable effect on the exoerythrocytic forms of *P. gallinaceum* (James and Tate, 1937; Raffaele, 1938): atabrine was without influence upon those of *P. relictum* (Rodhain, 1939). Kikuth and Mudrow (1939) studied the therapeutic effect of quinine, atabrine, "Certuna," and plasmochin on the exoerythrocytic forms of the virulent strain of *P. cathemerium*. They found that plasmochin was the only one of the four drugs which appeared to effect these stages directly.

Evidently, much more investigation is needed concerning the action of drugs on different stages of the parasite to establish the facts and clear up apparent discrepancies. The question of specificity of drugs for sporozoites, gametocytes and trophozoites is a fundamental problem. Are different kinds of drugs needed to produce cure of the acute infection, to prevent relapse, and to act as causal prophylactics?

Species and strain variation in susceptibility to drugs. It is stated that the species of parasites causing human malaria react differently to drugs. Thus, organic arsenic compounds are effective on *P. vivax*, but have no influence on *P. falciparum*; methylene blue acts strongly on *P. malariae*, very little on *P. vivax* and not at all on *P. falciparum*; quinine is most effective against *P. vivax*, but relapses from quinine are greatest with *P. vivax* and least with *P. falciparum* (Schulemann, 1932). It might be expected that differences would exist in the susceptibility to drugs of different species and even strains of avian malarial parasites. The differences in the response of various species of avian *Plasmodium* to drugs has been especially studied by Manwell (1930, 1932, 1934). He found that different strains of *P. relictum* reacted to treatment essentially the same whether quinine or plasmochin was used but that *P. relictum*, *P. rouxi*, *P. cathemerium*, *P. circumflexum*, and *P. elongatum* in the canary showed marked differences in susceptibility. Plasmochin was uniformly superior to quinine. but the degree of superiority depended on the species used. Kikuth (1931) also found that *P. relictum*, *P. cathemerium*, *P. elongatum*, and *P. circumflexum* varied in susceptibility to quinine and plasmochin. Manwell (1933) stated that atabrine does not prevent or cure infections with *P. cathemerium* or *P. circumflexum* but it was a perfect specific against infections with *P. rouxi*. Manwell and Haring (1938) also compared the effect of atabrine and plasmochin in *P. vaughani* infections in the canary. Rauwolfine is stated (Manwell, 1933–34) to have no action on *P. elongatum*, *P. relictum*, or *P. rouxi* infections in the canary, but to have a slight effect on those due to *P. cathemerium*.

Brumpt and Bovet (1936) compared the action of various drugs on the *Haemoproteus* infection of the finch as well as on an induced infection with *P. paddae* in the same animal. They correlated these results with data on *P. relictum* infections of canaries. Table 2 shows the results. Recently, Manwell, Counts

and Coulston (1941) found that sulfapyridine was without influence on infections caused by *P. relictum* and *P. nucleophilum*, but influenced that caused by *P. circumflexum* in the canary. Very little information has been published on differences in susceptibility to drugs of strains within a species. Lourie (1934) observed a considerable difference between two strains of *P. relictum* in canaries in their response to identical dosage schedules of quinine.

It is important to have more data in regard to species and strain susceptibility to drugs. What avian malarial species resembles most closely in its susceptibility to drugs the species producing infections in man? The answer to this question involves a study of the species and strain susceptibility to drugs of the parasites available for experimental infections and a comparison of the data obtained with what is known or can be found as to the action of the same drugs in the three types of human infections.

Can drugs completely cure avian infections? As stated above, the Sergents in their first fundamental work on the chemotherapy of avian malaria found that quinine did not cure consistently a *P. relictum* infection in the canary. Two

TABLE 2

DRUG	P. PADDAE	HAEMOPROTEUS	P. RELICTUM
Quinine	+	0	+
Atabrine	+	0	+
Plasmochin	+	+	+
710 F*	+	+	+
852 F†	0	+	+

+ = Definite therapeutic effect: 0 = No therapeutic effect.

* 8-Diethylaminopropylamino-6-methoxyquinoline.

† 8-Diethylaminoundecylamino-6-methoxyquinoline.

criteria of "cure" were introduced by these workers: namely, 1, no infection from injection of blood from the "cured" bird into a clean bird, and 2, reinfection of the "cured" bird with the same strain and species of parasite. The data of several investigators show that occasional canaries infected with *P. relictum* were sterilized by quinine (when the above criteria of cure are adopted). Manwell found that *P. cathemerium* infections were not sterilized, and Lourie (1934) stated that daily injections of quinine in these infections for nine months failed to sterilize the birds. Hegner and Manwell (1927) found that plasmochin failed to effect a "cure" in *P. cathemerium* infections. Manwell in a series of papers stated that plasmochin sterilized 100 per cent of canaries infected with *P. rouxi* and *P. elongatum*, about 25 per cent of those with *P. relictum* and *P. circumflexum* and none of those infected with *P. cathemerium*: quinine was much less effective as a sterilizing agent in all species.

Drug-fastness. No satisfactory evidence for drug-fastness has been presented. The Sergents (1921) described a quinine-fast strain in 1 canary with *P. relictum* and Kritschewski and Halpern (1933) stated that quinine-fast strains of *P. relictum* when passed through the mosquito sometimes lost and sometimes re-

tained their fastness. However, no good proof of fastness was given and Lourie (1935) in a careful investigation of the matter found no evidence of drug-fastness to quinine of *P. cathemerium*.

In vitro studies. Since a method of cultivating avian malarial parasites *in vitro* is not available, *in vitro* studies have not been extensive or satisfactory. The effect of mixing infected blood with quinine or other drugs and incubating for various lengths of time before injecting into clean birds is reported by several workers (Kopenharis, 1911; Hegner, Shaw and Manwell, 1928; Borchardt, 1930; Lourie, 1934). Borchardt incubated canary blood infected with *P. relictum* with a drug for 5 hours at 37°. Minimum concentrations which prevented infection when incubated blood was injected into canaries were: quinine, 50; hydroquinine, 400; and plasmochin, 20 mgm. per cent. Lourie (1934) found that *P. cathemerium* would resist 1 hour's incubation at 39° with 200 mgm. per cent of quinine. Anschütz (1910) found quinine and methylene blue produced on avian malarial organisms *in vitro* only slight microscopic changes. The recent study of Trager (1941) on the conditions affecting the survival *in vitro* of *P. lophurae* might be utilized with advantage in further studies of the *in vitro* action of antimalarials.

Kikuth (1938) has recently proposed a test for antimalarial activity in which the concentration of a drug which *in vitro* prevents exflagellation of male gametocytes is determined. He found that the results do not agree with those obtained with Roehl's method in canaries, but do agree with those found in the treatment of *Haemoproteus* infections in the finch.

Although the effect of drugs on the oxygen consumption of monkey malarial parasites *in vitro* has been studied (Fulton and Christophers, 1938) similar studies with avian malarial parasites are just beginning (Coggeshall and Maier, 1941). These preliminary studies indicated that inhibition of oxygen consumption *in vitro* alone cannot be depended upon to furnish an index of chemotherapeutic efficiency.

Mechanism of action of drugs. The difficulty of cultivating plasmodia *in vitro* is a definite handicap in investigations in this field. Quinine, and to a much lesser extent, plasmochin and atabrine, are the drugs which have been used in attempts to elucidate the mechanism of action in avian malaria. The simplest theory of mechanism is a direct plasmodicidal effect. Much higher concentrations of quinine than are ever obtained in the blood of treated animals appear to be necessary to kill the parasite *in vitro*. Roskin and Romanowa (1931, 1934) stated that ultraviolet rayed quinine was more effective than unrayed quinine, and that the organic arsenicals were no more effective when used with ultraviolet radiation than when used alone. These data might be used to support the idea of quinine being changed by the host to an active product, but there is no further evidence for this theory of action. The Sergents (1922) found that an extract of the spleen of a mammal treated with quinine had no therapeutic effect in avian malaria. Kritschewski and Demidowa (1934–35) stated that blockade of the reticulo-endothelial system with trypan blue decreased the effectiveness of quinine, plasmochin and atabrine. Oesterlin (1937) claimed that

dyes like methylene blue depend on the oxidation-reduction potential for action in bird malaria. Fischl and Singer (1934-35) stated that atabrine could be recognized in plasmodia by the fluorescent microscope. It has been claimed that active drugs are only found among those with a high partition coefficient for red blood cells (Hegner, Shaw and Manwell, 1928; Shaw, 1928). Fulton (1937) could not confirm this hypothesis.

Lourie (1934) found that quinine administered to canaries with *P. cathemerium* infections interfered with the normal growth and reproduction of the parasites and upset the characteristic synchronicity of the asexual cycle. These changes did not occur when the powerful influences of the body defenses brought about disappearance of parasites from the blood at the crisis. He, therefore, believed that quinine acted primarily on the parasite and not on body defenses. Boyd and Dunn (1939, 1941) also found that quinine, plasmochin and atabrine, when administered to canaries infected with *P. cathemerium*, inhibited the occurrence of reproduction and brought about a decrease in the size of merozoite group.

Although no conclusion as to the mechanism of action of quinine in avian malaria can be drawn from the scanty data available at present, it appears to the reviewer that Lourie's idea of a primary action of the drug on the parasite and not an action of the drug on body defenses will prove to be correct. This is almost certainly the mechanism of action of the sulfonamide drugs against bacterial infections (Marshall, 1941). It is to be noted that lower concentrations of these drugs are required for effective therapy *in vivo* than those required to kill bacteria *in vitro*. The intimate mechanism of the action of the sulfonamide drugs in duck malaria may be similar to their action in bacterial infections, because the action of these drugs was antagonized by p-aminobenzoic acid in both infections (Marshall, Litchfield and White, 1942).

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